Microplastics in Wastewater Treatment Systems and Receiving Waters

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Dedication

To Mom and Dad

Abstract

Plastic pollution is a problem of global scale and will increase as synthetic polymers continue to be produced, used, and discarded. Microplastic (MPs, <5 mm in size) pollution is of increasing concern, because this is estimated to account for more than 92% of global plastic counts and expected to present risks to aquatic fauna and humans. Often, MPs are too small to be seen and are unevenly distributed in the environment due to differences in shape, size, and density, rendering them difficult to find and quantify in environmental samples. Hence, adequate quantitative and qualitative assessment of these materials remains scarce, particularly in freshwaters and wastewaters that remain largely unexplored. However, both systems receive and transport different types of anthropogenic waste, including MPs, so warrant further attention for identification of mitigation strategies.

The purpose of this research was to fill gaps in knowledge of the role of freshwater rivers and wastewater treatment plants (WWTPs) as transport vectors of MPs to the environment, and generate incisive understanding of the distribution and behaviour of MPs in these systems. These research findings are expected to be relevant to stakeholders and regulators as they can aid in the identification of priority areas for further research, monitoring, and regulation of MP pollution. Therefore, this research focussed on the abundance and distribution of MPs (60-2800 µm) in urban fresh- and wastewater systems in a river whose catchment contains a large city: the River Clyde catchment (4000 km^2) in the city of Glasgow, Scotland. First, liquid fractions were sampled at eight treatment stage points within a tertiary WWTP with 184,500 population equivalents and receiving a mix of household and trade effluent. Then, sediment and water samples were collected in the recipient river, the River Clyde, upstream and downstream from the WWTP. In addition, sediment samples were collected from another nearby freshwater river, the River Kelvin, which also drains through Glasgow and the Clyde at its estuary. The overall aim of this research was to assess the extent of MP pollution in these systems and the distribution, transport, and possible fate of primary and secondary types of MPs. Microplastics were separated from their environmental matrix using the widespread protocols of density separation, hydrogen peroxide oxidation of labile organics, and filtration. Particles were identified by visual sorting followed by chemical confirmation of plastics.

Microplastics were ubiquitous and present in all water bodies in varying quantities: 161-432 MPs kg^{-1} in the River Kelvin tributary, <1-13 MPs L^{-1} in the WWTP, 1-26 MPs kg^{-1} in River Clyde sediment, and 0-4 MPs 24 L^{-1} in River Clyde water. The WWTP displayed high efficiency, removing 96% of incoming pieces, with the majority removed by the primary treatment stage. However, at least one fibre was observed in treated effluent and this may represent daily discharges of at least 12 million particles to the River Clyde from this WWTP. Total MP concentrations in sediment and water samples of the recipient river were higher in the most downstream site compared to the upstream point furthest from the effluent pipe. Fibre concentrations were higher in downstream sediment samples that may indicate some retention in rivers by sedimentation processes – this is supported by the high abundance of fibres in River Kelvin sediments. Fragments were abundant in the main river sediments in similar concentrations across upstream and downstream sites, suggesting these are more likely to be introduced from diffuse sources via surface runoff and in-stream transport. The comparable concentrations observed across sampling events for each of these systems suggest a continuous input of MPs from their source to the environment.

For further insight into the relative distribution of primary and secondary MPs and their potential sources, it is necessary to confirm material composition of these particles. A subset of specimens extracted from wastewater (5%), Clyde sediment (15%), and Clyde water (56%) were analysed by Fourier transform infrared spectroscopy (FTIR) for this assessment. Secondary MPs especially fibres were predominant, while primary MPs that have received the most media and public attention and prompted plastic and MP strategies, were lowest in concentration. Polypropylene (PP) was the most detected polymer across all analysed particles and was mainly present in the form of fibres and fragments. Polyester and nylon fibres that may be expected in high abundances in wastewater appeared absent in the WWTP in this study, although this was concluded mainly due to size limitations of the characterisation method. However, the PP fibres in wastewater could originate from sanitary products, medical applications, thermal clothing, and construction materials. This is important as fibres are often linked to washing machine effluent and currently little information from alternate sources for this type of MPs exists. In River Clyde sediment, fibres identified as polyethylene terephthalate were observed and concluded to originate mainly from fishing gear, based on combined assessment of chemical and visual properties.

Understanding the causes and significance of MP pollution is a new but expanding area of water research. It was important to share these research findings with the community and so this research was published when possible. This thesis is thus constructed from a series of published and unpublished papers.

Table of Contents

List of Tables

List of Figures

Preface

This thesis includes re-prints of the following papers that have been published in peer-reviewed journals:

Blair RM, Waldron S, Phoenix V, Gauchotte-Lindsay C (2017) Micro- and nanoplastic pollution of freshwater and wastewater treatment systems. Springer Sci Rev 5: 19-30 DOI: 10.1007/s40362-017-0044-7

[Included in **Chapter 2**]

Blair RM, Waldron S, Phoenix VR, Gauchotte-Lindsay C (2019) Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. Environ Sci Pollut Res DOI: 10.1007/s11356-019-04678-1
Pre-print (DOI: 10.31223/osf.io/vr9z7) available 7/6/18: https://eartharxiv.org/vr9z7/

[Included in **Chapter 4**]

Blair RM, Waldron S, Phoenix VR, Gauchotte-Lindsay C (2019) Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. Water Res DOI: https://doi.org/10.1016/j.watres.2019.114909
Pre-print (DOI: 10.31223/osf.io/ez5gm) available 8/3/19: https://eartharxiv.org/ez5gm/

[Included in **Chapter 5**]

The student is first and corresponding author for all publications. The co-authors listed in the papers are the student's supervisors. Authors' contributions to each paper are provided at the start of the relevant chapter.

The full published papers are included at the beginning of the corresponding chapters but sections and graphs have been re-numbered in accordance with University formatting guidelines.

Acknowledgements

I wish to express my deepest gratitude to my supervisors Professor Susan Waldron and Dr. Caroline Gauchotte-Lindsay, for the opportunity to work on this fascinating project, and for their support, guidance, and encouragement throughout my PhD programme. I would also like to thank Professor Vernon Phoenix for his supervisory role and contributions to research design during the earlier part of my research.

I gratefully acknowledge the Hydro Nation Scholarship Programme for funding this project, and for allowing me to be a part of the Hydro Nation family and making this an unforgettable experience in so many ways. To my fellow Hydro Nation scholars, thanks for the memories.

I owe a great deal of appreciation to Kenny Roberts for his unreserved help and continued support during the field and lab work at various stages of the PhD.

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This PhD research would not have been possible without the help and support of many people so to those I have not named individually, please know I am grateful for your contributions to the achievement of my research.

Finally, I am extremely grateful to my friends and family for their unconditional love and never-ending patience and encouragement during this long academic journey away from home. The distance has never been easy.

Mom and Dad, you mean the world to me and know that all my achievements are for you.

Declaration of originality

I certify that the thesis presented here for examination for a PhD degree of the University of Glasgow is solely my own work other than where I have clearly indicated that it is the work of others (in which case the extent of any work carried out jointly by me and any other person is clearly identified in it) and that the thesis has not been edited by a third party beyond what is permitted by the University's PGR Code of Practice.

I declare that the thesis does not include work forming part of a thesis presented successfully for another degree.

I declare that this thesis has been produced in accordance with the University of Glasgow's Code of Good Practice in Research.

I acknowledge that if any issues are raised regarding good research practice based on review of the thesis, the examination may be postponed pending the outcome of any investigation of the issues.

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Registration Number:

Abbreviations

Polymers

EVA	ethylene-vinyl acetate
PA	polyamide (nylon)
PBT	polybutylene terephthalate
Pest	polyester
PE	polyethylene
PET	polyethylene terephthalate
PMMA	polymethyl methacrylate
POM	polyoxymethylene
PP	polypropylene
PS	polystyrene
PTFE	polytetrafluoroethyelene (teflon)
PUR	polyurethane
PVC	polyvinyl chloride
PVS	polyvinyl stereate

Methods

ATR	attenuated total reflectance
BSE	backscattered electron
Cat 2 BSC	category 2 biological safety cabinet
DS	density separation
EC	electrical conductivity
EDS	energy dispersive spectroscopy
FPA	focal plane array
FTIR	Fourier transform infrared spectroscopy
H_2O_2	hydrogen peroxide
IC	inorganic carbon
NaCl	sodium chloride
NO^2	nitrite
NO^3	nitrate
OC	organic carbon
SE	secondary electron
SEM	scanning electron microscopy
SO4	sulphate
TEM	transmission electron microscopy
TOC	total organic carbon
WPO	wet peroxide oxidation

General

CEC	contaminant of emerging concern
CSO	combined sewer overflow
DO	downstream
EU	European Union
MBR	membrane bioreactor
MNP	micro- and nanoplastic
MP	microplastic
OM	organic matter
p.e.	population equivalents
POP	persistent organic pollutant
SEPA	Scottish Environment Protection Agency
SPRI	Scottish Pollutant Release Inventory
UK	United Kingdom
UP	upstream
USA	United States of America
WWT	wastewater treatment
WWTP	wastewater treatment plant

Names

RMB	Reina Maricela Blair
SW	Susan Waldron
CGL	Caroline Gauchotte-Lindsay
VP	Vernon Phoenix

1 Introduction

1.1 Background and rationale

The origin of plastics can be traced back to the early nineteenth century with the discovery of the process of vulcanisation by Charles Goodyear, by which sulphur and heat were applied to natural rubber to modify this into a more resistant and hardened material (Strong 2000). The use of vulcanised rubber was followed by the invention of other semi-synthetic plastics including cellulose-based materials like Parkesine, developed in Europe in the 1850s, and cellulose nitrate, developed in the USA in the 1860s (Brydson 1999; Strong 2000; British Plastics Federation 2014). A milestone for the modern age of plastics arrived in 1907 when Leo Hendrix Baekeland discovered the first stable thermoplastic known as Bakelite - this is considered the first fully-synthetic plastic and was and is still sometimes used today for electrical insulation (Brydson 1999; Strong 2000; British Plastics Federation 2014). Development of other synthetic polymers followed in the late nineteenth and early twentieth centuries, although initially some of these plastics were discovered by accident (Strong 2000). For example, polyethylene (PE), one of the most common types of plastics today, was discovered in 1933 when a leak in an experimental vessel allowed small amounts of oxygen to react with ethylene under high pressure, but researchers initially were unable to reproduce the process (Strong 2000). As scientists advanced their understanding of the synthesis process, new materials were developed in the early twentieth century, including polyvinyl chloride (PVC), polystyrene (PS), and nylon (Brydson 1999; Strong 2000). The onset of World War II prompted new demands for these materials and thus began the mass production of a wide variety of synthetic plastics and their moulding to meet different needs in everyday lives (Brydson 1999; Strong 2000).

The term plastic represents a wide array of synthetic polymers primarily made of carbon and derived generally from crude oil and natural gas (Strong 2000). Plastics can be separated into two main groups: thermoplastics and thermosets (Strong 2000; Plastics Europe 2018). Thermoplastics are solids at room temperature and can be melted, hardened, and reshaped repeatedly – these include commonly used materials like PE, polypropylene (PP), PS, PVC, and polyamides (PA) (Strong 2000; Plastics Europe 2018). Thermosets can be solids or liquids at room temperature, and are chemically altered when heated, thus cannot be re-melted and reshaped. Examples of thermosets are polyurethane (PUR), silicone, acrylic and epoxy resins (Strong 2000; Plastics Europe 2018). The popularity of plastics can be attributed to their unique characteristics such as their light-weight and durability that make them convenient and versatile for multiple applications across different industries, with some of the most common types of plastics used currently including PE, PP, PS, and PVC (Plastics Europe; **Table 1-1**).

Table 1-1 Densities and applications of some common polymers (derived from information available on the British Plastics Federation and Plastics Europe websites).

Polymer	Density (g cm^{-3})	Examples of use
Polypropylene	0.89	Packaging, toys, household appliances, lighting diffusers, CD cases, fishing lines
Polyethylene	0.96	Packaging, plastic bags, bottles, bullet-proof vests
Acrylonite-butadine-styrene	1.05	Sports equipment, toys, car parts
Polystyrene	1.06	Packaging, household appliances, consumer electronics, disposable medical items, building and construction
Polyamide	1.14	Textiles, fishing lines, carpets, food packaging
Polycarbonate	1.21	Bottles, CDS and DVDs, food containers, eyeglass lenses
Cellulose acetate	1.3	Eyeglass frames, toothbrushes, tool handles, wrapping
Polyester	1.37	Textiles, ropes, insulation, plastic bottles
Polyvinyl chloride	1.39	Building products, piping, coatings, low-voltage insulation, packaging, medical and leisure products
Polyethylene terephthalate	1.39	Engineering plastics, external building parts

Plastics are a part of the modern world and have become intrinsic to daily activities, thus their production has continuously increased since their discovery. Global plastic production has grown from 1.7 to 348 x 10^6 metric tons in the 1950s and 2017, respectively, with nearly half dominated by the packaging sector (Geyer et al. 2017; Plastics Europe 2018; **Figure 1-1**). The fast increase in plastic production looks set to continue and has been accompanied by a rapid generation of plastic waste, mainly in the form of thermoplastics like low and high density PE, PP, and PA fibres (Geyer et al. 2017). As plastics are designed to be durable, much of what was produced since the start of their mass production is still around today and has resulted in a cumulative generation of 6.3 x 10^9 metric tons of plastic waste from 1950 to 2017 (Geyer et al. 2017), and this has likely increased in the last two years. Approximately 79% of this waste has been discarded in landfills and the natural environment, and some may eventually reach the oceans (Geyer et al. 2017), where it comprises 60-90% of all marine debris (UNEP and GRID-Arendal 2016). The accelerated generation of plastic litter and inadequate disposal of this waste have created a global crisis that can lead to serious negative impacts. One of the more visible consequences is wildlife entanglement in larger debris like fishing nets, plastic bags, and packaging remains, although some less visible effects have also been noted as a result of ingestion or uptake of smaller particles. However, plastic waste can occur in different forms, each with different implications and some of which may still be unknown.

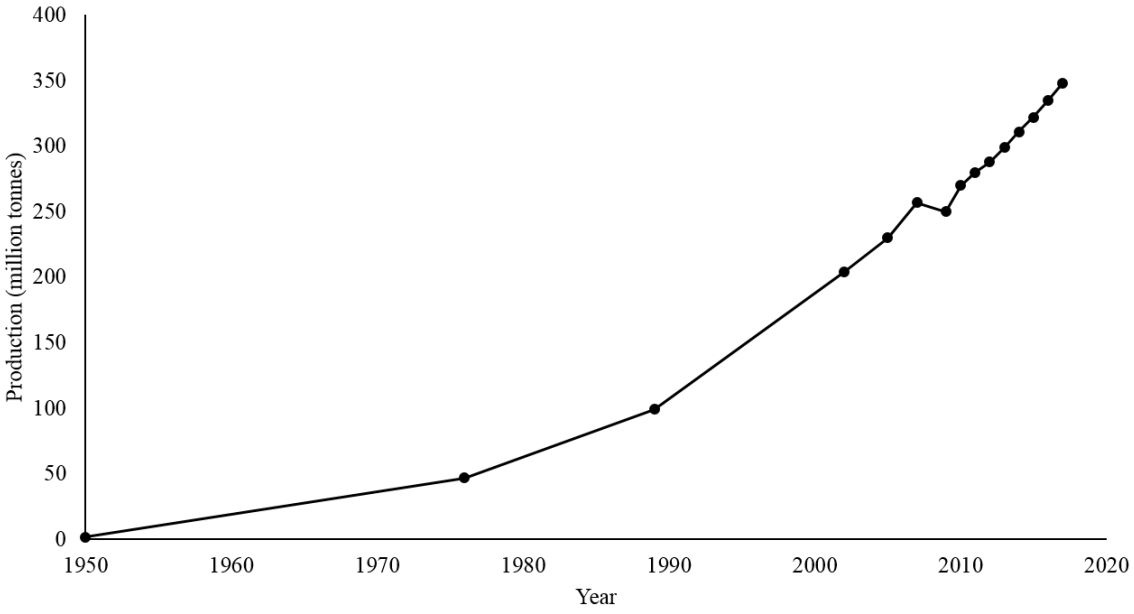

Figure 1-1 World plastic production from 1950 to 2017 (contains data from © Plastics Europe: includes thermoplastics, polyurethanes, thermosets, elastomers, adhesives, coatings and sealants, and polypropylene-fibers; does not include the following fibers: PET-, PA-, PP- and polyacryl-fibers).

Plastic waste can originate from different sources and thus occur in different shapes and sizes. In general, plastic debris has been classified according to size into macroplastics, mesoplastics, microplastics, and nanoplastics, but different definitions have been proposed for each category (**Figure 1-2**). Microplastics (MPs) that are the focus of this research, have generated increasing attention from the media and the public in recent years, and initially were mainly associated with microbeads and pre-production pellets (Carpenter et al. 1972). Currently, MPs are generally recognised as particles smaller than 5 mm (Arthur et al. 2009; GESAMP 2015), but agreement on a formal definition is yet to be reached. Recent evidence suggests that MPs are ubiquitous, present even in remote locations, and estimated to account for ~92% of global plastic counts (Eriksen et al., 2014). Microplastics include a wide array of highly-variable materials, making it difficult to characterise their behaviour as a single category. However, their unique combination of physical and chemical properties separates them from other microparticles in various aspects. For example, unlike natural microparticles, MPs are specifically designed to be durable so they will take longer to degrade, they are highly resistant and buoyant so can be easily transported over long distances, and different MPs have been noted to host unique bacterial assemblages (Arias-Andres et al. 2018). Furthermore, MPs exhibit heterogeneous distribution, unlike the homogeneous distribution observed for other microparticles such as engineered nanoparticles in wastewaters (Lambert and Wagner 2018). However, more recent studies suggest that MPs transport in rivers may resemble that of sediment and low-density particles, thus propose their retention and release could be predicted by fine-tuning transport models for these materials (Kooi et al. 2018).

Microplastics are recognised as contaminants of emerging concern (Hartl et al. 2015) because they are presumed harmful to wildlife and humans. For example, MPs can contain other toxic substances

that can be added during manufacturing (e.g. plasticisers, flame retardants, pigments) or collected during their passage through the environment (e.g.PCBs, metals, pathogens) (Andrady 2011; Lambert and Wagner 2018). Furthermore, their small size and physical appearance render them more accessible for accidental or intentional ingestion by organisms, thus possibly exposing them to these toxins (Andrady 2011). However, their toxicity is not fully understood as MP abundance is subject to uncertainty due to differences in methods for their quantification and for measuring risk of exposure.

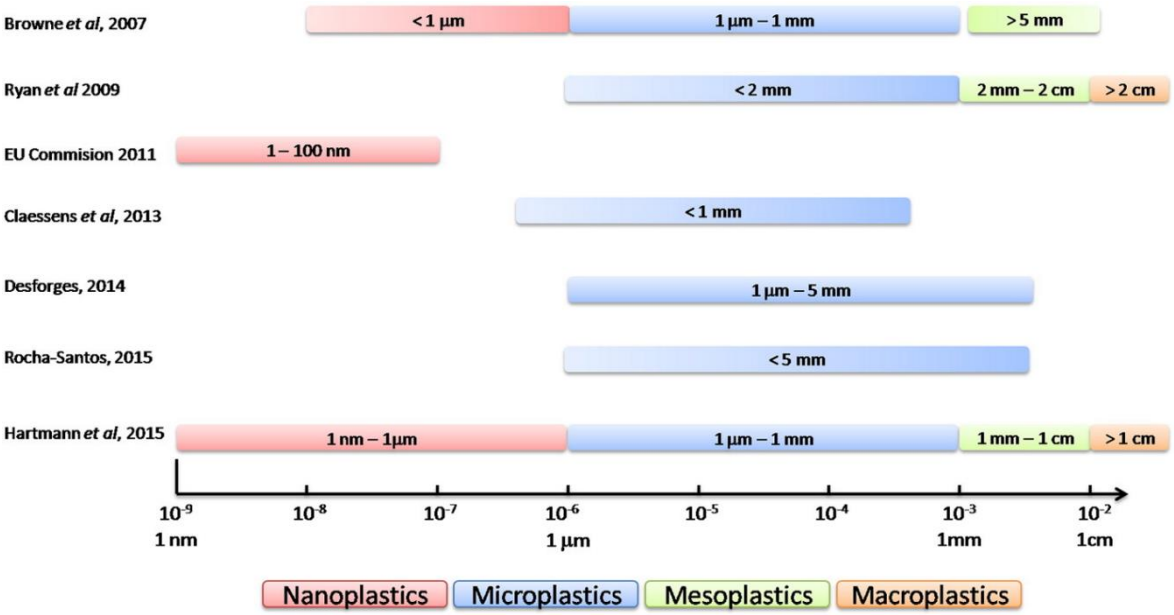

Figure 1-2 Size-based definition of plastics proposed by different authors (Figure 1 in Da Costa et al. 2016).

Microplastic research started in oceans, as this is where they were first observed (Carpenter et al. 1972). In 2004, a paper published in Nature demonstrated that plastic fragments and fibres were also widespread in oceans, and called for further research to understand their occurrence and consequences (Thompson et al. 2004), triggering great interest in this topic and a rapid increase in the number of publications (Blair et al. 2017). Despite the rapidly expanding body of literature, MPs research is challenging because these contaminants are often too small to be seen and include a wide array of contaminants that are highly variable in shape, size, and density, rendering them difficult to isolate and measure. Moreover, standardised definitions, units of measure, and protocols for extraction and characterisation of MPs are not yet available, and quantification of smaller MP fractions (e.g. <20 µm) in environmental compartments is still missing. Furthermore, their uneven occurrence in the environment complicates their investigation, as there is no guarantee that field samples are representative of spatial and temporal variations and that all possible MPs in the sample are being extracted in each study. Hence, adequate quantitative and qualitative assessment of these materials in aquatic systems remains limited, and more research is needed to assess the extent of their pollution and severity of their threat.

Microplastics will travel from their source to rivers via diffuse and point sources like WWTPs, and from source to oceans via rivers. However, research in MPs is biased towards oceans and very little

is understood about rivers and wastewater treatment plants (WWTPs) that are important transport routes of anthropogenic pollution in the environment (**Figure 1-3**). Understanding of MP pollution in these systems is a crucial gap in knowledge that provides the underlying motivation for this study. Specifically, investigation of MPs in WWTPs and their receiving waters may provide incisive understanding of transfer of MPs from land to oceans and help to identify strategies to reduce their inputs. Therefore, this PhD research focussed on the presence, distribution, and movement of MPs from their inflow to sewage systems, to their discharge to lotic freshwaters, contributing to understanding the role of WWTPs and rivers as conduits or filters of these contaminants.

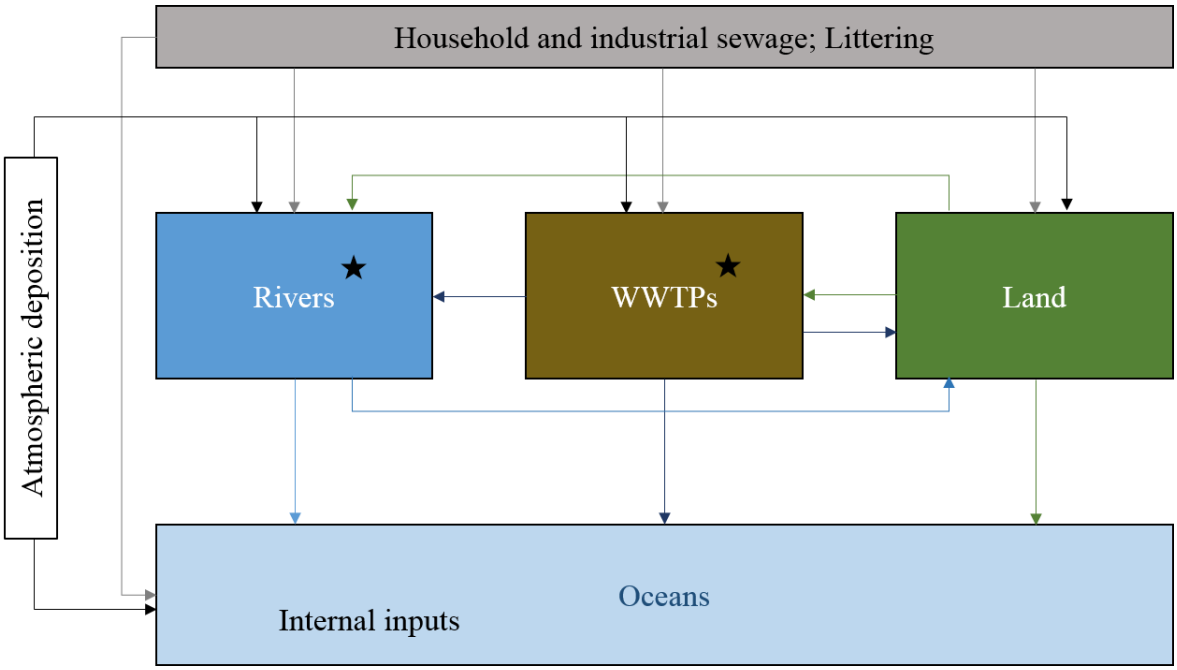

Figure 1-3 Generalised diagram of potential flow paths of MPs from land-based sources to oceans. ★ Pathways explored in this PhD project.

1.2 Research aims

The overarching aim of this study is to assess the prevalence and distribution of MPs in WWTPs and freshwater rivers, in order to assess their potential sources and retention and release, and to inform regulators on possible remediation strategies.

The specific research objectives were to:

1) Quantify the main types of MPs present in sewage treatment systems and freshwater rivers in an urban catchment
2) Assess the ability of sewage treatment to process MPs, and estimate average loading to and from the WWTP
3) Assess the potential retention and release of MPs in the recipient channel and explore possible contributions of point and diffuse sources of MPs to rivers

1.3 Thesis structure

The specific research aims were investigated through two main campaigns centred on examination of (1) an advanced wastewater treatment facility, and, (2) a large freshwater river receiving sewage discharges from the WWTP, in an urban catchment. A short study was also conducted during the first year at a smaller freshwater river that is a tributary to the larger river system at its estuary. The preliminary study in the smaller river was necessary to guide the experimental design and planning of subsequent work at the advanced WWTP and its recipient channel, as well as provide data from an independent system for comparison.

The work presented in this thesis describes the results of these three research studies that form the PhD project, and how their outcomes advance knowledge on the topic of MPs pollution in wastewater and freshwater environments. Several components of this thesis have been published and so the thesis is structured in a paper format with these publications forming chapters where appropriate. At the beginning of each chapter, there is a description of research attribution and whether the chapter has been expanded. The thesis is composed of six chapters structured as follows:

Chapter 2 provides a review of the literature, relevant to the theoretical and conceptual framework for this PhD project and formed the basis of the Blair et al. 2017 publication. While this paper reflects the understanding of knowledge in 2017, MP research has been expanding, and new and relevant literature has become available in the past two years. Further, the author's understanding on the sources, occurrence and fate of MPs in these systems has evolved through this research, some of which is documented in two further papers (Chapters 4 and 5), which include an updated literature review for freshwaters and WWTPs, respectively. An addendum is included at the end of the chapter to expand the literature review specific to freshwater rivers relevant to the Chapter 6 discussion. Based on the collective messages from the reviewed literature, the hypothesis testing framework is introduced in the final section of this chapter to support the general and chapter-specific objectives.

In **Chapter 3**, an overview of the methods is introduced, but the specific methodology for river sediment and wastewater samples is detailed in the following empirical chapters. This chapter begins with an overview of the study sites located in the River Clyde catchment, and the types of samples collected for each. General protocols followed for extraction of MPs from different matrices are then discussed briefly to avoid reiteration with later chapters and summarised in table form. This chapter finishes with added discussion on the theory for identification techniques including visual sorting by light microscopy and chemical characterisation using scanning electron microscopy coupled with energy dispersive spectroscopy (SEM-EDS) and Fourier transform infrared attenuated total reflectance spectroscopy (FTIR-ATR), as these are only briefly introduced in the literature review in the previous chapter.

The research work for the PhD project begins in the River Kelvin, a freshwater tributary to the main river site, located in the west end of Glasgow, UK. This part of the study is comprised of two sampling campaigns in December 2015 and February 2016 to generate the first MP dataset for the project. Here, the focus was on (1) obtaining an initial profile of MP types expected in the river catchment, and (2) calibrating sampling, extraction, and identification techniques to optimise

measurement of the different types of MPs. This work was summarised in Blair et al. 2019a, which forms **Chapter 4**.

The River Kelvin data provided information on possible MP types in the catchment, thus the next aim of the research was to assess the contributions of different sources in urban catchments, first by examination of known point sources of anthropogenic contaminants, specifically WWTPs. Thus, **Chapter 5** contains the research conducted during the second and third years of PhD, focussing on spatio-temporal sampling of the liquid wastewater fractions at multiple stages of the treatment process in a tertiary WWTP in Glasgow. Sampling for this part of the project was carried out at Daldowie WWTP over a 10-month period. Here, the focus was on assessing (1) average daily incoming and outgoing flows of MPs, and, (2) the stepwise effect of advanced treatment on MP concentration in a tertiary WWTP in an urban catchment. The understanding generated is summarised in Blair et al. 2019b, currently published and available as a preprint online at EarthArXiv since March 2019. The full manuscript in its final version is included at the beginning of the chapter with additional data provided as an addendum on colour repartition for Daldowie and complementary data for two activated sludge samples obtained from Shieldhall, a nearby secondary WWTP in the same river catchment.

The final part of the research is described in **Chapter 6** and was to (1) investigate the spatiotemporal distribution and characteristics of MPs, (2) infer the potential contributions of MPs from the WWTP and other diffuse and point sources of pollution, and (3) assess the potential retention and release of different types of MPs. This uses spatial sampling of sediment and surface water in the River Clyde, the recipient channel to Daldowie WWTP and the River Kelvin. As the River Clyde is in close proximity to the ocean environment, this research can provide insight into the discharge of marine MPs from in-land waters. Understanding of the sources and retention and release of MPs by riverine systems can inform integrated catchment management strategies for remediation of MP pollution. This chapter is written in the traditional format for a thesis chapter.

The thesis concludes with **Chapter 7** consisting of a summary of the main findings, according to the specific objectives of this project. This chapter further reflects on methodological challenges, the relevance of this study to advance knowledge on MPs pollution in aquatic environments, and recommendations for future work.

2 Literature review: Micro- and nanoplastic pollution of freshwater and wastewater treatment systems

Springer Science Reviews
DOI 10.1007/s40362-017-0044-7

ANALYTICAL STUDENT REVIEW

Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems

Reina M. Blair[1] · Susan Waldron[1] · Vernon Phoenix[2] · Caroline Gauchotte-Lindsay[3]

Received: 15 December 2016 / Accepted: 9 May 2017

Abstract Plastic waste is a widespread and persistent global challenge with negative impacts on the environment, economy, human health and aesthetics. Plastic pollution has been a focus of environmental research over the past few decades, particularly in relation to macroplastics that are easily visible by the naked eye. More recently, smaller plastic waste at the micro- and nanoscale has become of increasing concern, resulting in extensive investment in research to advance knowledge on the sources, distribution, fate and impact of these materials in aquatic systems. However, owing to their small sizes and a lack of unified methods, adequate quantitative and qualitative assessment has been difficult. Furthermore, most of the microplastic surveys available to date have focussed in the marine environment while scarce knowledge exists of freshwater systems. Because the majority of marine debris originates on land, the role of wastewater treatment systems and natural fluvial vectors in delivering these emerging contaminants to the environment should be explored. Considering fundamental aspects pertaining to microplastic sources, distribution, mobility and degradation in these systems is crucial for developing effective control measures and strategies to mitigate the discharge of these particles to the sea.

Keywords Plastic pollution · Microplastic · Freshwater · Wastewater · Nanoplastic · Emerging contaminants

Endorsed by Susan Waldron.

Electronic supplementary material The online version of this article (doi:10.1007/s40362-017-0044-7) contains supplementary material, which is available to authorized users.

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Introduction

Plastic waste is pervasive and increasing in land and water environments globally. The 2013 global plastic production was estimated to be 299 million tonnes, a 3.9% increase from 2012 [77]. Germany and the United Kingdom (UK) are the two highest producers of plastic waste in the European Union (EU), recovering 80 and 26% of it, respectively [46]. Most plastic in the environment is non-biodegradable and remains as waste for a long time [29], with approximately 10% ending up in the oceans [90]. Plastics are lightweight and buoyant, and easily transported long distances across a wide range of environments [15], rendering them ubiquitous contaminants. Previous research from shoreline and beach surveys across all continents indicates that plastic waste commonly accounts for 50–90% of all marine litter [21]. About 80% originates from land-based sources [2, 15, 41], suggesting fluvial systems are important transport routes of these contaminants to the sea. However, compared to marine systems, data for freshwaters remain limited, and the magnitude of their impact has yet to be assessed [24].

The emphasis on plastic pollution research in oceans until recently may be because its accumulation and impacts appeared to be more evident in these environments [84]. For example, patches of accumulated floating macroplastic

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Statement of authorship

RMB researched, gathered, and reviewed the data sources included in this report, wrote the original manuscript, and prepared and submitted the paper to the journal. SW, VP, and CGL contributed to the editing of the manuscript. This paper was accepted for publication without further reviewers' comments. An addendum written by RMB is included at the end of the published material as an update to the literature review specific to freshwater rivers, relevant to Chapter 6. Additionally, general and chapter-specific hypotheses formulated by RMB based on the above theoretical foundation, are introduced.

Chapter introduction

The purpose of this chapter is to summarise the current body of knowledge relevant to the theoretical and conceptual framework on MP pollution in freshwaters and WWTPs and the methods used for their assessment.

Citation

Blair RM, Waldron S, Phoenix V, Gauchotte-Lindsay C (2017) Micro- and nanoplastic pollution of freshwater and wastewater treatment systems. Springer Sci Rev 5: 19-30 DOI: 10.1007/s40362-017-0044-7

Abstract

Plastic waste is a widespread and persistent global challenge with negative impacts on the environment, economy, human health and aesthetics. Plastic pollution has been a focus of environmental research over the past few decades, particularly in relation to macroplastics that are easily visible by the naked eye. More recently, smaller plastic waste at the micro- and nano-scale has become of increasing concern, resulting in extensive investment in research to advance knowledge on the sources, distribution, fate, and impact of these materials in aquatic systems. However, owing to their small sizes and a lack of unified methods, adequate quantitative and qualitative assessment has been difficult. Furthermore, most of the microplastic surveys available to date have focussed in the marine environment while scarce knowledge exists of freshwater systems. Because the majority of marine debris originates on land, the role of wastewater treatment systems and natural fluvial vectors in delivering these emerging contaminants to the environment should be explored. Considering fundamental aspects pertaining to microplastic sources, distribution, mobility and degradation in these systems is crucial for developing effective control measures and strategies to mitigate the discharge of these particles to the sea.

2.1 Introduction

Plastic waste is pervasive and increasing in land and water environments globally. The 2013 global plastic production was estimated to be 299 million tonnes, a 3.9 % increase from 2012 (Plastics Europe 2015). Germany and the United Kingdom (UK) are the two highest producers of plastic waste in the European Union (EU), recovering 80 % and 26 % of it, respectively (Hartl et al. 2015). Most plastic in the environment is non-biodegradable and remains as waste for a long time (European Commission DG Environment 2011), with approximately 10 % ending up in the oceans (Thompson 2006). Plastics are lightweight and buoyant, and easily transported long distances across a wide range of environments (Coe and Rogers 1997), rendering them ubiquitous contaminants. Previous research from shoreline and beach surveys across all continents indicates that plastic waste commonly accounts for 50-90 % of all marine litter (Derraik 2002). About 80 % originates from land-based sources (GESAMP 1991; Coe and Rogers 1997; Andrady 2011), suggesting fluvial systems are important transport routes of these contaminants to the sea. However, compared to marine systems,

data for freshwaters remains limited, and the magnitude of their impact has yet to be assessed (Eerkes-Medrano et al. 2015).

The emphasis on plastic pollution research in oceans until recently may be because its accumulation and impacts appeared to be more evident in these environments (Ryan et al. 2009). For example, patches of accumulated floating macroplastic debris were observed in gyres and convergence areas in oceans over a decade ago (Moore et al. 2001; Ryan et al. 2009; European Commission 2011). Furthermore, the marked mechanical effects of plastic litter on marine biota due to entanglement and ingestion raised concerns of its potential harm to biodiversity and ecosystems (Derraik 2002; Ryan et al. 2009; Thompson et al. 2009). While oceans have been used as waste dumps for years despite global efforts to prevent this (Gordon 2006), the majority of plastic litter is produced inland, thus examining its transport to marine environments by rivers may allow for identification and regulation of its main sources (Ryan et al. 2009; Dris et al. 2015).

At present, the increased awareness of the growing production and accumulation of plastic pollution in the environment has brought greater focus to the need for development of policies and management strategies. For example, the United Nations Environment Programme (UNEP), called for an urgent need to address plastic pollution of oceans through implementation and enforcement of coordinated strategies, effective policies and regulations, campaigns, and other incentives at national, regional and global levels (Jeftic et al. 2009). The European Marine Strategy Framework Directive (MSFD) 2008/56/EC emphasised the need for more data on the amount, distribution, and composition of plastic debris (Galgani et al. 2011; Sadri and Thompson 2014). However, despite the extensive research devoted to monitoring plastic debris over the past decades, the full extent of its quantity, distribution, and impact remains widely unknown. Thus, controlling plastic waste may be confounded by lack of measurement of the extent and thus understanding of source and impact, rather than strategy (Coe and Rogers 1997). Further, the importance of plastic fragments at the micro- and nanoscale has only recently been recognised, and method development to define and measure them is still under way. Microplastics and nanomaterials have been classified by Scotland's Centre of Expertise for Waters (CREW) as emerging contaminants, or alternatively, "contaminants of emerging concern" (CEC) for Scottish watercourses, due to their toxic characteristics and the lack of adequate data for reliable risk assessment (Hartl et al. 2015). Therefore, it is essential to refine the initial estimates of plastic debris in oceans and inland waters to include these smaller and "invisible" fractions, and identify their main sources before further actions or regulations be implemented.

The concepts of micro- and nano-sized plastics as emerging contaminants, and the role of wastewater and freshwater systems as sinks or sources of these materials to the environment provide the focus for this review. This review synthesises the theory and literature relevant to the topic of micro- and nanoplastic pollution in freshwaters and wastewater systems, including methods for their examination, and identifies knowledge gaps and areas where further investigation are needed.

2.2 Micro- and nanoplastics

Plastic litter can occur in a wide range of sizes. The literature commonly distinguishes between two broad classes of plastics: macroplastic (>5 mm) and microplastic (< 5 mm) (Arthur et al. 2009;

Thompson et al. 2009; Faure et al. 2012; GESAMP 2015), but different terms and size ranges have been used across studies (**Table 2-1**). A unified lower limit for measurement for microplastics has not been defined, but for practical purposes 333 µm (~0.3 mm) is often used when sampling with neuston nets (Arthur et al. 2009; Roex et al. 2013). Nevertheless, because a lower cut-off has not been established, the term microplastic has often been used to encompass pieces ranging from millimetre to nanometre dimensions. More recently, the term "nanoplastic" has been introduced as a separate category (Besseling et al. 2014; Wagner et al. 2014; Koelmans et al. 2015). This size class has been defined as particles smaller than 0.2 mm based on the WG-GES size classification (Wagner et al. 2014), and, smaller than 100 nm according to the general definition used for nanomaterials (Koelmans et al. 2015). Mostly, nanoplastics have been overlooked in the literature and are the least-studied size class, as evidenced by a lack of discussion of its definition and quantification. Nevertheless, it has been suggested that these nanoscopic plastics may be the most hazardous yet due to their high potential for bioaccumulation and biomagnification (Koelmans et al. 2015; Pinto da Costa et al. 2016), thus requiring further investigation. For purposes of this review, for ease of reference the rest of the discussion will focus on micro- and nanoplastics (MNP) jointly as one single size class.

Table 2-1 Size class definition of aquatic plastic debris used by various authors.

Preffix	Size Class	Size Range	Source
Nano	nano, micro, millimetre (NMM)	not available	Besseling et al., 2014 (abstract)
	Nanoplastic	< 0.2 mm	Wagner et al., 2014
		< 100 nm	Koelmans et al., 2015
Micro	Microliter	~0.06 - 0.5 mm	Gregory and Andrady, 2003
	Microplastic	< 0.5 mm	Thompson et al., 2004 Fendall and Sewell, 2009 Hoellein et al., 2014 (abstract) Sanchez et al., 2014 Corcoran et al., 2015
		0.333 - 5 mm	Arthur et al., 2009
	micro debris	< 2 mm	Lechner et al., 2014
	small microplastic	< 1 mm	Vianello et al., 2013
		0.2 - 1 mm	MSFD Technical Subgroup on Marine Litter, 2013
		> 0.3 mm (< 1 mm)	Faure et al., 2015
	large microplastic	1 - 5 mm	MSFD Technical Subgroup on Marine Litter, 2013 Faure et al., 2015
Meso	Mesolitter	> 0.5 mm	Gregory and Andrady, 2003
	meso debris	5 - 25 mm	MSFD Technical Subgroup on Marine Litter, 2013
		2 - 20 mm	Lechner et al., 2014
		> 5 mm	Sanchez et al., 2014
Macro	macroplastic/ macro debris	> 25 mm	MSFD Technical Subgroup on Marine Litter, 2013
		20 mm	Sanchez et al., 2014
		> 5 mm	Faure et al., 2015
Mega	mega debris	100 mm	Sanchez et al., 2014

2.2.1 Sources

Micro- and nanoplastics are classified into two general categories according to source: primary and secondary. Their source of origin determines their shape and composition. Primary MNP are intentionally manufactured in small sizes for different applications, including personal care and cleaning products, and pre-production pellets for fabrication of other plastic goods (Thompson et al. 2004; Arthur et al. 2009; Ryan at al. 2009; Roex et al. 2013; Storck et al. 2015). The manufacture of primary nanoplastics will likely increase with their use in electronic devices, medicines, cars, and airplanes (Roex et al. 2013). Primary MNP are likely to be collected mostly intact in industrial and household sewage, and go through wastewater treatment (WWT) facilities before being discharged into the aquatic environment (Roex et al. 2013). Secondary MNP originate from the breakdown of larger plastic pieces due to weathering by UV-radiation and physical defragmentation by mechanical forces (Thompson et al. 2004; Arthur et al. 2009; Cole et al. 2011). Thus, macroplastics will breakdown into microplastics, and these will further break down into nanoplastics. Their abundance

and production rates will depend on environmental characteristics and polymer type (Williams and Simmons 1996; Thompson et al. 2004; Arthur et al. 2009; Ryan at al. 2009; Dubaish and Liebezeit 2013), making secondary MNP input to oceans harder to trace, quantify, and control than primary sources.

Chemical composition, size and surface features of MNP can provide insight to their origins. For example, primary MNP found in personal care products tend to be smaller than 0.3 mm, contain additives (e.g. plasticisers), and are composed mainly of PE, but also may contain PP, polyethylene terephthalate (PET), polymethyl methacrylate (PMMA) and Teflon (PTFE) (Roex et al. 2013; Storck et al. 2015). Primary MNP in the form of pre-production pellets will be mainly spherical or cylindrical around 5 mm in size (GESAMP 2015). The polymers PE, PP, and PS are often used in packaging and thus are indicative of urban origins, while denser polymers like PVC and polyester (Pest) are commonly used in construction and textiles, respectively (GESAMP 2015). These plastics will be introduced as secondary MNP fragments and fibres from sewage effluent or surface runoff (Sadri and Thompson 2014). Currently, there are no data in the literature on the relative abundances of primary to secondary MNP, and only a couple of studies have examined the relationship between fragments of different sizes (Lee et al. 2013). Thus, there is a need to address these knowledge gaps for accurate quantification of MNP fractions, assessment of the relationship among abundances of different size classes, and application of precise source characterisation approaches for understanding the potential contributions of different urban and industrial sources (Lee et al. 2013). This information is crucial for managing this problem and informing policy, since it is predicted that even if land-based inputs are controlled, plastic debris densities in oceans will continue to increase from secondary sources (Eerkes-Medrano et al. 2015).

2.3 Microplastics in freshwater environments

Microplastics were described in the literature as early as the 1960s and 1970s (GESAMP 2015), but it was not until 2004 that the term became widely used (Thompson et al. 2004). Although plastic litter is not a new problem, only recently have MNP become a focus of the scientific community with publications on the topic increasing rapidly (Faure et al. 2012; GESAMP 2015), particularly in marine systems (see reviews by Andrady et al. 2011 and Cole et al. 2011). Data on MNP pollution of continental freshwaters are less abundant than for marine systems (**Figure 2-1**; Thompson et al. 2009; Wagner et al. 2014, Eerkes-Medrano et al. 2015), but the number of publications are also increasing, mostly since 2014. The research published between 2011 and 2014 on microplastics in freshwater bodies in Asia, Europe, and North America has been reviewed (Eerkes-Medrano et al. 2015), and additional studies of American, Asian, European, and African sites have been published in 2015 and 2016 . All surveys report the presence of different size classes of plastics in these environments and, where available, high relative abundances of MNP compared to macroplastics, in both sediment and surface waters.

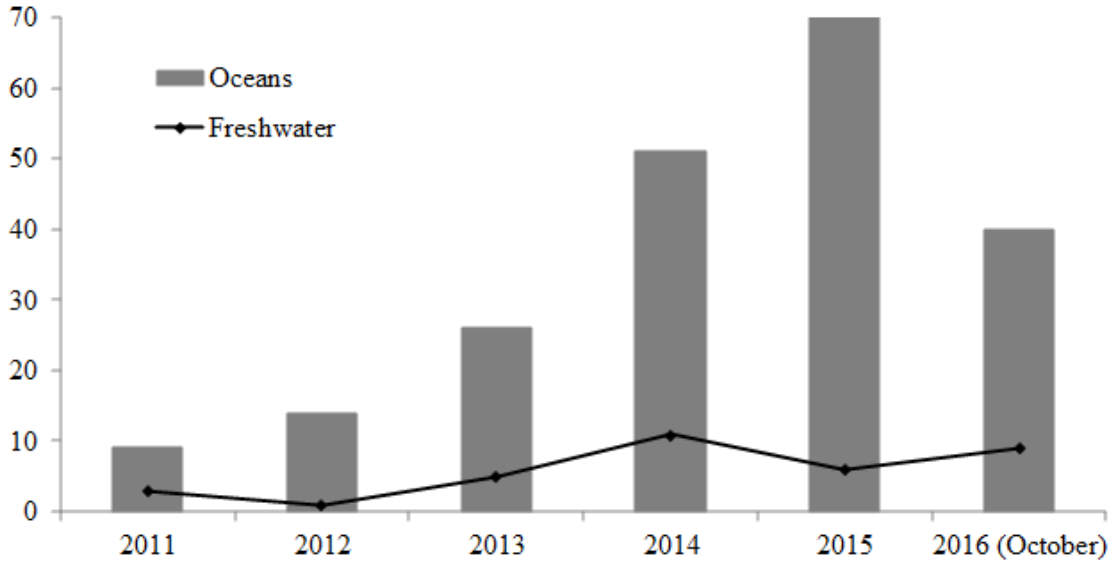


Figure 2-1 Comparison of marine vs freshwater microplastics literature published between 2011 – September 2016, based on Web of Knowledge search engine accessed 3/10/2016 for search words: "microplastic + freshwater" and "microplastic + ocean".

In America, most research has concentrated in Canada and the USA, (**Figure 2-2** and **Table A-1**), particularly in the Great Lakes area (Zbyszewski and Corcoran 2011; Eriksen et al. 2013; Rios-Mendoza and Evans 2013; Zbyszewski et al. 2014; Corcoran et al. 2015; Ballent et al. 2016), including the St. Lawrence River watershed (Castañeda et al. 2014). Of these, only two studies collected data from freshwater bottom sediments (Castañeda et al. 2014; Corcoran et al. 2015), and one considered open-water loading (the Laurentian Great Lakes system; Eriksen et al. 2013). The rest of the studies focussed on lakeshore surveys. Across these studies, microplastics were present in both sediment and surface waters, in higher densities compared to macroplastics, and with a high predominance of pellets and fragments, indicative of contributions from both primary and secondary sources. In addition, microbeads found in the St. Lawrence River were comparable in size, shape and composition to those found in the Laurentian Great Lakes (Castañeda et al. 2014), indicating a possible transport of these materials from the municipalities along the river to the lakes. A more recent study in the Palisades Reservoir and Snake River in Idaho, USA, reported microplastics in 72% of the samples consisting mostly of films and fibres (McDevitt et al. 2016), suggesting a greater contribution from secondary sources.

In Asia, (**Figure 2-2** and **Table A-1**) a study in Lake Hovsgol, Mongolia, reported average pelagic microplastic densities of 20,264 items km^{-2}, despite its remoteness and low population density (Free et al. 2014). As microplastic abundance would be expected to be relatively lower in such areas, this was attributed to the lake's long residence time, small surface area, and lack of proper waste management (Free et al. 2014), indicating a strong need for effective control measures. Similarly, a more recent study of remote lakes in China found evidence of microplastics in abundances of 8 ± 14 – 563 ± 1,219 items m^{-2} and attributed their presence to riverine inputs to the lakes and to a lesser extent atmospheric transport (Zhang et al. 2016). Data from remote areas are rarely generated but are important for understanding the ubiquity of these materials, as well as their transport pathways and

fate. However, it remains necessary to consider developed areas with high industrial and anthropogenic activities. This is especially crucial in the Asian continent as the region contributes considerably to the global plastic production (Plastics Europe 2015). Marine data from the '90s indicate that plastic litter in the Japanese coast increased by a factor of 10 every 2-3 years (Ogi and Fukumoto 2000). Further, microplastic pollution has been reported in coastlines of Japan (Browne et al. 2011) and Korea (Lee et al. 2013; Kang et al. 2015) and in urban estuaries in China (Zhao et al. 2015). In this context, the region may present useful opportunities for studying these plastic particles in freshwaters that have highly populated and industrialised catchments, but the recent literature considering this is limited. In the Taihu Lake in China, microplastic abundances were highest in the most heavily-contaminated areas of the lake, and abundances observed in plankton net samples were the highest reported worldwide, from 0.01×10^6 to 6.8×10^6 items km^{-2} (Su et al. 2016).

The rest of the literature reviewed here between 2011-2016 comprises one study from Africa and several studies across Europe: Switzerland (Faure et al. 2012, 2015), Italy (Imhof et al. 2013; Vianello et al. 2013, Fischer et al. 2016), France (Dris et al. 2015), Germany (Dubaish and Liebezeit 2013; Wagner et al. 2014; Klein et al. 2015; Mani et al. 2015), Netherlands (Besseling et al. 2014), Austria (Lechner et al. 2014), and the UK (Morritt et al. 2014; **Figure 2-2** and **Table A-1**). In the African Great Lakes, suspected plastics were isolated from the gastrointestinal tracts of 55% and 35% of perch and tilapia samples respectively (Biginagwa et al. 2015). While total abundances were not provided, and water or sediment samples were not examined, the study provides the first evidence of microplastic presence in inland waters in the African continent and the only one to date. Similarly to the African study, Switzerland and Italy surveys have focussed on lake systems, with microplastics reported in Lake Geneva (Faure et al. 2012; 2015), the Lagoon in Venice (Vianello et al. 2013), and Lakes Garda (Imhof et al. 2013), Bolsena and Chiusi (Fischer et al. 2016).

Most of the earlier freshwater research appears to have focussed on lentic systems (i.e lakes), but rivers and WWT environments are gaining more attention as potential conduits of microplastics to the environment. A French study conducted in urban Paris sites was unique in being the first to quantify atmospheric fallout (Dris et al. 2015). The same study also collected wastewater and surface water of urban rivers and reported a predominance of fibres across the different systems. In Germany, microplastics in the form of fragments, granules, and fibres were reported in all sediments of the Rivers Rhine, Elbe, Mosel, Neckar, and Main (Wagner et al. 2014; Klein et al. 2015; Mani et al. 2015), and the Jade system of the southern North Sea (Dubaish and Liebezeit 2013). Similarly, the studies conducted in Netherlands, Austria and the UK, also considered free-flowing waters from the Rivers Dommel (Besseling et al. 2014), Danube (Lechner et al. 2014), and Thames (Morritt et al. 2014), respectively, and found evidence of microplastic pollution in all of them. Their findings support the consideration that these systems can be important transport routes but their distribution, retention and loading are largely influenced by a combination of in-stream processes and catchment characteristics.

Figure 2-2 Spatial distribution of microplastic studies conducted worldwide in freshwater and wastewater treatment (WWT) systems between 2011-September 2016 (n = 34), according to system type (lake, river, WWT or a combination of two or more of these).

2.3.1 Rivers as transport pathways of MNP

Rivers are dynamic systems that can either retain or transport MNP but quantitative evidence of river retention and discharge rates remains limited. It is considered that rivers can act as temporary sinks, delaying the release of microplastics to oceans, while transport of these materials can quickly increase during rain events due to increased flow rate (Galgani et al. 2000; McCormick et al. 2014; Rech et al. 2014). In Brazil (Araujo and Costa 2007; Ivar and Costa 2013) the presence of solid waste on beaches, including plastics, was attributed to domestic sources along the river basin, influenced by the proximity of river sources, and increased river flow during high rain events (Rech et al. 2014; Sadri and Thompson 2014). Similarly, the Danube River was identified as an important transport route of plastics from production sites in Germany and Austria to the Black Sea, and it was proposed that variations in floating densities were linked to release of plastics from nearby production facilities (Lechner et al. 2014). In Chicago, higher MNP densities were observed after rain events during wet periods for two urban rivers, with evidence of higher abundances of primary MNP that are not regulated by total maximum daily loads and being discharged into oceans (McCormick et al. 2014). However, export patterns are not always so clear. For example no major trends in particle sizes of larger plastic pieces (size categories not defined) was observed from up- to downstream sites in the Thames river, although generally higher abundances were observed in sites near sewage discharge (Morritt et al. 2014). The Tamar river in the UK was not determined to be a source of microplastics,

despite their high abundance (Sadri and Thompson, 2014), considered to reflect drainage of a largely unpopulated catchment (Eerkes-Medrano et al. 2015).

2.4 Microplastics in wastewater treatment systems

The relationship between population density and urban and industrial activities with MNP presence in aquatic environments can be explored via analysis of wastewater effluent from treatment facilities and receiving waters. The WWT process may not completely remove MNP (Browne et al. 2011; Eriksen et al. 2013; Eerkes-Medrano 2015; Storck et al. 2015), thus the role of each treatment stage in the degradation, generation, transport, and removal of MNP, particularly those originating from primary sources, should be considered. Conversely, as the systems are expected to function properly in order to minimize treatment costs and ensure adequate water quality standards, the impact of MNP in the treatment process should also be evaluated.

There is increasing focus on considering the relationship between effluent discharge of MNP from wastewater treatment plants (WWTP) and MNP abundances in the recipient channel (Hoellein et al. 2014; McCormick et al. 2014; Dris et al. 2015; Estahbanati and Fahrenfeld 2016). Generally, higher microplastic concentrations were observed downstream of WWTP relative to upstream - based on observations in the North Shore Channel in Chicago (Hoellein et al. 2014: McCormick et al. 2014) and in the Raritan river in New Jersey (Estahnabanti and Fahrenfeld 2016), USA. However, no upstream to downstream evolution was observed in Urban sites in Paris (Dris et al. 2015).

Loadings from WWTP and the removal efficiency of various treatment stages has also been considered (Chaskey et al. 2014; Dubaish and Liebezeit 2013; Magnusson and Nóren 2014; Carr et al. 2016; Murphy et al. 2016; Buksa and Niekerewicz 2016). For example, in New York, discharges of 109,556, 81,911, and 1,061,953 particles day^{-1} were reported for three different WWTP (Chaskey et al. 2014), while an average annual discharge of 9×10^8 particles was reported from a WWTP in Germany (Dubaish and Liebezeit 2013). In a smaller plant in Langeviksverket in Lysekil, Sweden, serving ~12,000 population equivalents, although most of the microplastics entering the WWTP were measured to be retained in the sewage sludge, the plant continued to discharge MNP - interpreted from higher concentrations in the recipient water compared to the reference site upstream (Magnusson and Nóren 2014). Similarly, recent studies conducted in WWTP in Glasgow (Murphy et al. 2016) and Southern California (Carr et al. 2016) observed that treatment was efficient in retaining microplastics via grit and grease removal (Glasgow) and skimming and settling processes (California). However, in both studies, secondary treatment plants continued to discharge microplastics at yields of 1 item 1.14×10^3 L^{-1} and 0.25 + 0.04 items L^{-1} (equivalent to 65 million items a day) in the Glasgow and California studies, respectively. It is possible that larger WWTP will contribute larger MNP loads, and thus an additional treatment step before discharging effluent to receiving waters may help reduce its MNP concentrations. This projection is based on the observation of few to no microplastics in tertiary outflow of a WWTP in Southern California (Carr et al. 2016). However, the general absence of quantitative studies considering removal at each stage of the treatment process makes this an area of high priority for further MNP research.

2.5 Ecological impacts

The ecological effects of MNP in freshwater systems has received some scrutiny (see review by Eerkes-Medrano et al. 2015), however this is limited. Owing to their small size, MNP can be ingested directly and indirectly by aquatic species more readily than larger particles, sometimes when mistaken for food, and leading to harmful physical effects (Derraik 2002). Evidence from marine studies for example, indicates that MNP ingestion may lead to choking, blocked digestive tracts, damage to organs, debilitation, and ultimately death (see review by Derraik 2002). Similarities in MNP ingestion by freshwater organisms to marine fauna has been observed (Imhof et al. 2013; Rochman et al. 2013; Sanchez et al. 2014; Biginagwa et al. 2015; see review by Eerkes-Medrano et al. 2015), but there is yet little evidence of uptake by fish and bird species in lakes (Faure et al. 2012, 2015).

In addition, MNP can adsorb persistent organic pollutants (POPs), potentially introducing toxicity throughout the food web (Mato et al. 2001; Endo et al. 2005; Bakir et al. 2012; Rios-Mendoza and Evans 2013), which could eventually reach humans by bioaccumulation (Farrel and Nelson 2013). Desorption of POPs and other manufacturing additives can increase pollutant concentrations in waters and increase the susceptibility of the larger pieces to degradation (Dubaish and Liebezeit 2013). Nevertheless, information on sorption and leaching of POPs from microplastics is scarce (Arthur et al. 2009), and most of the knowledge on toxicity derives from marine and laboratory experiments (Eerkes-Medrano et al. 2015), while data from freshwaters remains limited. Further, MNP surfaces can provide habitats for microbial colonisation and biofilm formation, allowing for migration of opportunistic pathogens and invasive species (Zettler et al. 2013, McCormick et al. 2014). The latter may be relevant for WWTP as it could affect the functioning of the treatment processes, as well as increase the transport of WWT bacteria from these facilities to receiving waters (Zettler et al., 2013; Tagg et al., 2015).

2.6 Methods for studying MNP

Micro- and nanoplastic research is still a developing field, with as yet no standardised procedures for their study, and method advancement is still in its early stages (Eerkes-Medrano et al. 2015; Tagg et al. 2015). The different size class distinctions and methods used may reduce comparability of results across studies, highlighting the need to unify size class definition and develop simple, low-cost, and precise methods for their detection and monitoring (Galgani et al. 2013; Eerkes-Medrano et al. 2015). However, it may still be too early to do so, as we have yet to identify the spectrum, sizes, and types of MNP that require greater attention; thus for now, standardised procedures may prove useful only in situations that call for regular site-specific monitoring or have limited budgets (Free et al. 2014; Eerkes-Medrano et al. 2015). Nevertheless, reviews of methods for identification and quantification of MNP in marine environments are available (Hidalgo-Ruz et al. 2012; Qiu et al. 2016), and the NOAA Marine Debris Program published a manual on recommended laboratory methods for quantifying plastics in oceanic waters and sediments (Masura et al. 2015). The methods used for freshwater systems are similar to those implemented in marine studies.

The review of methods presented here is based on the generalised pathway used across studies (**Figure 2-3**) and includes the techniques predominantly mentioned in the literature (**Table A-1**), tailored to gather information for quantification and characterisation of MNP, as well as describe their behaviour and fate in WWT and fluvial systems.

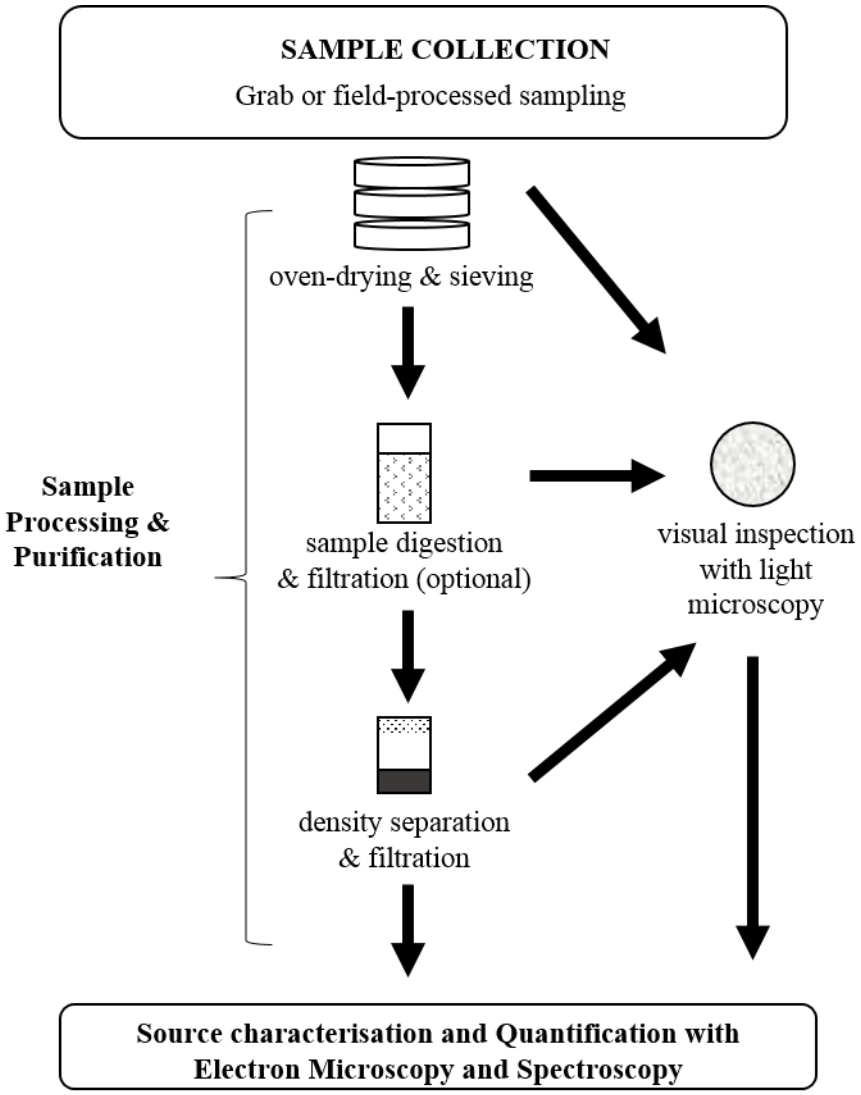


Figure 2-3 Generalised pathway for extraction and identification of microplastics from sediment and water samples in freshwater systems, based on a review of different methods employed by various authors.

2.6.1 Sampling and processing

Traditional sampling techniques for both surface water and sediments are common. Surface waters are often sampled using manta trawls and neuston nets (Hidalgo-Ruz et al. 2012), while both in situ filtration and bulk sampling have been described for effluent discharge collection (Browne et al. 2011; Hidalgo-Ruz et al. 2012; Chaskey et al. 2014; Magnusson and Nóren 2014; Murphy et al. 2016). For lake sediments, selective sampling of visible pieces from beach transects was a frequent practice, and grab-sampling equipment (e.g. Ekman, Van Veen, Peterson, and Ponar grabs) has been

used for collection of lake-bottom sediments (Hidalgo-Ruz et al. 2012; Castañeda et al. 2014; Corcoran et al. 2015). Shoreline sediment collection is generally accomplished through bulk sampling approaches such as steel trowels and box corers (Zbysewski and Corcoran 2011; Zbysewski et al. 2014; Vianello et al. 2013; Fischer et al. 2016).

Sample processing usually involves a combination of approaches including visual pre-selection, size fraction sieving, flotation and density separation, filtration, and organic matter (OM) digestion (Hidalgo-Ruz et al. 2012; Cole et al. 2014; Tagg et al. 2015; Qiu et al. 2016). Sieve analysis is useful for separation of particles into different size ranges. A wide range of sieve sizes has been used across studies, and this approach will largely determine the minimum sizes of plastic debris that are collected and quantified (Hidalgo-Ruz et al. 2012). For example, higher MNP abundances are usually reported where smaller mesh sizes were used in sieving and filtration (Hidalgo-Ruz et al. 2012; Storck et al. 2015). This is important as it may reduce the comparability and accuracy of results, possibly underestimating abundances in some cases from loss of material that is not retained in sieves and is discarded.

2.6.2 Sample purification

After physical sorting by sieving, samples are purified using flotation and density separation of MNP from the organic and inorganic medium. Here too there is variation. Most commonly, sodium chloride (NaCl) saturated solution is used for flotation of low-density particles from sediment (Hidalgo-Ruz et al. 2012). Sodium iodide (NaI) and sodium polytungstate (SPT) have been used to float polymers with higher densities, although this approach tends to be more costly (Claessens et al. 2013). However, the approach is the same across studies: the sample is mixed with the solution, shaken for a certain amount of time, and left to settle so that the lower-density particles rise to the surface. The floating pieces can be manually removed, and the smaller ones can be extracted by filtration of the supernatant through membrane filter (Hidalgo-Ruz et al. 2012). The filtered samples are then either visually inspected for identification of microplastics, or further purified with acid, alkaline or enzymatic digestion methods.

Wet digestion protocols have been commonly employed to disintegrate biological materials and facilitate the extraction of microplastics from organic-rich media. Numerous methods are available for chemical removal of organic matter (Mikutta et al. 2005) using different reagents such as H_2O_2 (Robinson 1927), NaOCl (Anderson 1963), $Na_2S_2O_8$ (Meier and Menegatti, 1997), HNO_3 in combination with H_2O_2 (Huang and Schulte 1985), and H_2SO_4 (Dean 1999). Selection of the adequate protocol is largely dependent on reaction conditions and sample-specific properties, but protocols employing H_2O_2 remain more widely used. The efficiency of protocols for removing organic material, with minimum impact on composition of microplastic pieces, using H_2O_2 (Claessens et al. 2013; Nuelle et al. 2014), HNO_3 (Claessens et al. 2013), NaOH (Claessens et al. 2013; Cole et al. 2014; Nuelle et al. 2014), HCl (Cole et al. 2014; Nuelle et al. 2014), HNO_3 in combination with HCl or H_2O_2 (Claessens et al. 2013), and enzymatic digestion (Cole et al. 2014), has been tested. The HNO_3, H_2O_2, and Proteinase-K enzyme techniques exhibited high performance in disintegration of OM, but their efficiencies seemed to rely largely on sample composition and reaction conditions (e.g., reagent concentration, temperature, and digestion time). For example,

HNO_3 removed more OM than H_2O_2, NaOH, and in combination with other reagents (Claessens et al. 2013), but these tests were performed on animal tissue only and direct digestion of PS spheres with HNO_3 altered their composition of PS spheres. Conversely, the application of 35% H_2O_2 digestion for seven days dissolved more organic debris than acids and alkalis, with minimal change to PP and PE particles (Nuelle et al. 2014). However, biogenic material <1 mm was not removed completely, and the remaining material was bleached, resulting in discolouration that could potentially interfere with visual identification of microplastics. The enzymatic digestion with Proteinase-K appears a rapid and efficient method to digest OM with ease, generating higher digestion efficacy (>97%) than acid and alkaline digestion in plankton-rich samples and copepod tissue, with no visible impact to microplastics (Cole et al. 2014). No tests have been conducted for OM removal efficiencies from wastewater or sludge samples using these approaches.

Alternative approaches for isolation of microplastics from sediment samples based on principles of elutriation (i.e. using a gas or liquid upward stream to separate particles) (Claessens et al. 2013; Kedzierski et al. 2016) and pressurised fluid extraction (Fuller and Gautam 2016) have been tested as a means to improve extraction efficiencies and showed promising results.

2.6.3 Characterisation and quantification

After initial sorting and separation, suspected MNP are characterised and quantified for assessment of spatial and temporal distributions (Hidalgo-Ruz et al. 2012). Typically, millimetre-sized particles are inspected initially under light microscopy, grouped according to different categories (e.g. type, shape, colour), and counted. Larger pieces are often counted with the naked eye or under a stereo microscope and identification of smaller pieces is commonly accomplished with the use of forensic techniques such as electron microscopy and spectroscopy techniques.

Electron microscopy provides further insight on the chemical and morphological characteristics of the plastic particles. There are two types of electron microscopy: scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Typically, suspected MNPs are analysed with a SEM coupled with energy-dispersive X-ray (EDS) microanalysis to produce backscatter electron (BSE) images and spectra for determination of elemental composition. This data can be used to discriminate carbon-based materials such as plastics from non-polymers as the plastics are made of C and so show C-specific signals different than non-plastic materials. While SEM appears to be employed often (Zbyszewski and Corcoran 2011; Eriksen et al. 2013; Imhoff et al. 2013; Vianello et al. 2013; Hoellein et al. 2014; McCormick et al. 2014; Zbyszewski et al. 2014) no studies reported using TEM.

Similarly, spectroscopic tools may be used for added analysis of individual particles, with Raman and Fourier Transform-Infra Red (FTIR) spectroscopy used more frequently. These techniques are applied to gather information on polymer type, and the crystalline structure of the particles, which may provide insight into the sorption behaviour of persistent, bioaccumulating, and toxic substances, and the degradation of MNP from changes in bond distances (Gerrard and Madams 1986). Here, the basic principle is that infrared radiation is passed through a sample, where it is absorbed, reflected or transmitted. Although there are few differences between techniques, the end result is a molecular

fingerprint represented by absorption and transmission, and as different materials will generate different spectra based on their unique molecular structures, the compound from which the MNP is derived can be identified (Das and Agrawal 2011). This information may be used to trace sample origin and is crucial in understanding site-specific loadings. The FTIR is gaining more popularity perhaps due to being non-destructive, less costly and easier to use, and involving little sample preparation (Tagg et al. 2015).

Spectroscopy methods can be combined with microscopy to improve accuracy of the results. For example, the combined use of micro-FTIR and molecular mapping by focal plane array (FPA), can help to reduce scanning time, and facilitate the analysis of entire membrane filters and smaller pieces without affecting spatial resolution (Vianello et al. 2013; Tagg et al. 2015). While the implementation of forensic techniques is becoming more common in more recent papers, these can be time-consuming and may not be accessible in every case. Therefore, use and selection of these approaches appears dependent largely on sample size and logistic constraints.

2.6.4 Modelling of transport

Whilst every freshwater and wastewater survey conducted to date has reported microplastic occurrence in water and sediment samples, total and relative abundances are highly variable among studies and even within studies where different zones of a water body have been considered. While this may be attributed partly to differences in sampling, extraction, and identification techniques, site-specific characteristics (e.g. morphology, surface and catchment area, wind patterns; Fischer et al. 2016) are likely to play an important role in MNP distribution and cycling in these systems. Higher abundances may be expected in habitats that accumulate smaller particles of sediment (Browne et al. 2011), and their distribution may be influenced by sediment transport and deposition processes (Castañeda et al. 2014; Vianello et al. 2013; Klein et al. 2015). Hydrodynamic effects can have greater influence in MNP distribution than population density, industrial activities, or sewage discharge and MNP concentrations in river shores, as observed in a case study in Germany (Klein et al. 2015). As such, transport models can be useful tools to simulate MNP behaviour in riverine systems and evaluate the factors that control their transport and distribution, However little focus has been given yet to investigate modelling approaches (Nizetto et al. 2016). To examine riverine and wastewater inputs, sources and flow or discharge can be used as with other contaminants to predict MNP loading. In the Danube, plastic load at mean flow, and a correction factor for population density, were used to calculate plastic inputs to the Black Sea (Lechner et al. 2014). Flow rate data from two California rivers were also used to estimate yields of >2 billion particles over a 72 hour period (Moore et al. 2011). In Venice, high correlations were observed between small microplastics and fine grain size, indicating both follow similar sinking and accumulation processes, with higher accumulation of MNP in low energy sites (Vianelllo et al. 2013).

Physical drivers for sediment transport can be tested to build models for MNP transport and storage, and identify areas of high deposition (Nizzetto et al. 2016). Fundamentally, if plastics behave in the same way as sediment, available hydraulic models can be easily applied to MNP load studies, and if they behave differently, the models can be fine-tuned to get their behaviour in properly. For example, use of a modified INCA-Contaminants simulator, utilising catchment hydrology, soil erosion and

metereological controls for prediction of microplastic accumulation and distribution, revealed strong hydrolological controls in transport and storage of microplastics (Nizzetto et al. 2016). Heavier and larger microplastics (>2.0 mm) were more likely to be retained in hotspots for sediment deposition, but high flow events caused their remobilisation (Nizzetto et al. 2016). A similar approach can be used with other available models, for example the Delft hydraulics model (Delft 3D suite) for rivers and estuaries. This model allows particle tracking and has a morphology module that predicts sediment movement (Deltares, https://oss.deltares.nl/web/delft3d/about). Statistical approaches could explore linkage between model parameters e.g., the relationship between grain and MNP size classes, and later incorporated into the transport model to project loading of MNP from freshwaters systems. Further research should focus on modelling approaches as a tool to predict MNP fate in the environment and further understanding of the inheritance of terrestrial MNPs to oceans.

2.7 Conclusions

This analysis contributes to recent freshwater and methodology reviews (Hidalgo-Ruz et al. 2012; Eerkes-Medrano et al. 2015; Qiu et al. 2016) by widening the on-going discussion to include the more recent publications, wastewater treatment surveys, and additional methodological approaches that can generate incisive understanding of key aspects of MNP pollution in these systems.

Micro- and nanoplastic fragments originating from primary or secondary sources are contaminants of emerging concern (GESAMP 2015; Hartl et al. 2015). Considerable work undertaken in recent years advances knowledge of MNP contamination of aquatic environments, but several key challenges remain in this new field of water research. With the majority of surveys to date focussing on marine systems, further research should aim to expand spatial coverage of MNP studies, especially for continental waters, and consider the role of free-flowing freshwaters as transport vectors of land-based inputs to oceans, especially those receiving discharges from WWTP. A few WWT studies are available, but these systems remain largely understudied, providing an area for further investigation. Further, studies conducted in WWT systems should consider not only the removal of MNP by treatment processes, but also the impact of these contaminants on the efficacy of the treatment plant, and their potential for picking up and transporting substances and bacteria that may jeopardise water quality in the recipient channel.

Owing to their small sizes and a lack of unified methods, adequate quantitative and qualitative analysis and reliable risk assessment of MNP has been difficult (Hartl at al. 2015), especially in the case of nanoplastics, which are yet to be isolated from environmental samples. A unification of methodology for improved quantitative and qualitative assessment of the microplastic fractions could provide guidance for examination of nanoplastic fractions, which are believed to increase in importance as an ecological threat in coming years (Gigault et al. 2016). While a wide array of protocols have been tested for MNP evaluation, method development research should consider using sample purification and forensic techniques in combination rather than individually, and aim to unify size class definitions and units of measurement to improve comparability among studies. In fluvial systems, modelling tools can be useful to assess key aspects regarding transport, degradation, storage, and fate of MNP in the environment. These considerations will result in a more accurate assessment

of MNP abundance and distribution, both in inland and oceanic waters, helping to reduce errors in reporting results, and contributing to identification of where control measures should be implemented.

2.8 Addendum

Research on MPs in freshwater systems has expanded since the start of the PhD project in 2015, and since concluding the first river experiment in 2016. The literature search was stopped in February 2019, at which time a total of 29 published freshwater river studies were identified from 2011 until 2019 (**Table A-2**). A few of these studies were reviewed in Chapter 2, but as the majority were published between 2017 to present, an overview is presented in this section specifically for papers focussing on MPs pollution in freshwater river environments.

Freshwater river studies vary by location and methods (**Table A-2)** but all studies have observed widespread distribution of MPs, often in large concentrations in sediment and water compartments, as well as in their aquatic fauna. The research for these systems seems to concentrate primarily in the American continent with most studies located in the USA and Canada (n=9). In these North American studies, various Great Lakes tributaries have been considered (Castañeda et al. 2014; Baldwin et al. 2016; Ballent et al. 2016; Vermaire et al. 2017), allowing researchers to explore possible linkages between riverine concentrations and those reported previously in the Great Lakes (Eriksen et al. 2013; Zbyszewski et al. 2014). For example, sediments in the St. Lawrence River in Quebec, Canada, contained an average abundance of 13,759 microbeads m^{-2} (Castañeda et al. 2014), comparable to levels in the Great Lakes (Eriksen et al. 2013), while the Ottawa River, a major tributary to the St. Lawrence, contained lower concentrations that averaged 220 MPs kg^{-1} (Vermaire et al. 2017). In a different study, concentrations across Lake Ontario tributaries ranged from 20 to 27,830 MPs kg^{-1} and exhibited high variability at a small spatial scale, but these were generally lower than those in nearshore sediments of the recipient lake (Ballent et al. 2016). On the US side of the Great Lakes, surface water samples collected from tributaries contained between 0.05 and 32 MPs m^{-3} (Baldwin et al. 2016), and while it is difficult to compare these concentrations with the other Great Lake studies as different matrices were sampled, they are comparable to abundances in US river systems in Chicago (1.9-17.9 m^{-3}, McCormick et al. 2014; 2.3-5.7 m^{-3}, McCormick et al. 2016). Therefore, for the American continent, most of our understanding comes from these North American studies, and Latin America was, until recently, an unexplored area in MPs research. However, new information from a 2019 study in the Atoyac River basin in Mexico (Shruti et al. 2019) suggests that this region may be subject to higher pollution levels. Sediment samples collected in the Zahuapan and Atoyac Rivers contained 1633 and 1133 MPs kg^{-1}, similar to concentrations observed in the Valquesillo Dam that receives water from these rivers (Shruti et al. 2019), but comparably higher than those reported in other locations. Nevertheless, further research is needed to explore other water systems in this part of world and inform policy, particularly as several countries in Latin America may lack recycling laws.

In 2015, Asian studies were rare in the literature, but there has been a recent spike in research in this continent, most studies located in China and one more current study from Japan (**Table A-2**). The Three Gorges Dam system in China, including the Yangtze River and other tributaries has received special attention (Zhang et al. 2015; Wang et al. 2017b; Zhang et al. 2017; Di and Wang 2018). In

the Yangtze River, for instance, average MP concentrations in surface waters ranged between 2516 and 4703 MPs m^{-3} (Wang et al. 2017b; Di and Wang 2018). A different study in the Yangtze reporting different units of measure observed pollution levels of 3407.7 x 10^3 to 13,617.5 x 10^3 MPs km^{-2}, which were higher than those in other tributaries assessed in the same study (192.5 x 10^3 to 11,889.7 x 10^3; Zhang et al. 2015), but lower than the Xiangxi River (0.55 x 10^5 to 342 x 10^5 MPs km^{-2}; Zhang et al. 2017), one of the main tributaries to the Yangtze. Generally, sediments in the Three Gorges tributaries were lower than in surface waters, with 80 to 864 MPs m^{-2} in the Xiangxi River (Zhang et al. 2017) and 82 ± 60 MPs kg^{-1} in the Yangtze (Di and Wang 2018). Studies elsewhere in China examined sediments from the Beijiang River (Wang et al. 2017a) and different Shanghai rivers (Peng et al. 2018), where levels ranged from 178 to 802 MPs kg^{-1}, indicating more advanced pollution than the Three Gorges tributaries. While Chinese waters seem to exhibit some of the highest concentrations reported in freshwaters worldwide, comparably lower pollution levels were observed in Japan. The first survey of Japanese waters provides datasets for 36 sites across 29 rivers, sampled between August 2015 and May 2018 (Kataoka et al. 2019). In Japan, MPs were widespread across all rivers with an average pollution level of 1.6 MPs m^{-3}, and their composition and size distribution were similar to those reported in East Asian seas (Isobe et al. 2015), which may suggest some outflow of MPs from inland waters to the surrounding sea (Kataoka et al. 2019).

Recent studies have also advanced datasets for European freshwaters in Netherlands, (Leslie et al. 2017), Germany (Leslie et al. 2017; Schmidt et al. 2018), the UK (Horton et al. 2017; Hurley et al. 2018), and Portugal (Rodrigues et al. 2018), where MP abundances are variable. The recent evidence corroborated earlier observations on the widespread distribution of MPs in the River Rhine, for example, where concentrations of 1700 to 4900 MPs kg^{-1} were measured in suspended particulate matter, mostly spheres smaller than 300 µm (Leslie et al. 2017). These concentrations differed from those observed in the nearby River Meuse where a total of 1400 MPs kg^{-1} were present in suspended particulate matter, mainly in the form of fibres (Leslie et al. 2017). Furthermore, MPs were also observed in urban canal waters and sediment in Amsterdam, with mean abundances of 100 items L^{-1} and 2071 items kg^{-1}, respectively (Leslie et al. 2017). The first dataset for freshwater rivers in the UK was obtained from sediment samples in the River Thames tributaries (River Leach, River Lambourn, and The Cut), where MPs were found at all sites with an average of 180 to 660 items kg^{-1} (Horton et al. 2017). Moreover, higher concentrations were observed in more polluted sites that had a predominance of fragments, while fibres were more abundant in other sites (Horton et al. 2017). Similar to the River Thames system, MPs were widespread in the Rivers Irwell and Mersey catchments in the UK (Hurley et al. 2018). Microplastics were observed in 39 out of 40 sites in both rivers and 5 accumulation hotspots were identified - these had an average of 34,500 particles kg^{-1} (Hurley et al. 2018), representing the highest concentrations reported in European waters. In Portugal, seasonal variation in MPs abundances were observed, and generally, MP levels were one to two orders of magnitude higher than those reported in most European sites (Rodrigues et al. 2018), but comparable to those in the Thames (Horton et al. 2017) and the Beijiang (Wang et al. 2017b) rivers. Concentrations in the Antua River were 58 to 193 and 71 to 1265 MPs m^{-3} in March and October water samples, respectively, and 100 to 629 and 18 to 514 MPs kg^{-1} in March and October sediment samples, respectively, suggesting some seasonal variations.

Seasonal changes in MP abundances in water and sediments can provide insight on the fate of MPs in rivers related to hydrology and sediment dynamics. Overall, rapid changes have been observed in MP concentrations during runoff events in American, UK, and South African, and Japanese rivers (Baldwin et al. 2016; Hurley et al. 2018; Nel et al. 2018; Kataoka et al. 2019). For example, in US Great Lake tributaries, MP concentrations were higher in surface water during runoff events (Baldwin et al. 2016). In the UK, MP concentrations in sediments of the Irwell and Mersey fell after severe flooding in some sites, indicating that extreme flooding events exported ~70% of MPs, equivalent to 43 billion particles (Hurley et al. 2018) and supporting the observations from the Great Lakes. Furthermore, the South African study found that sediment MP concentrations were higher in July compared to February, likely attributed to low energy and higher sediment deposition due to reduced river flows during the summer (Nel et al. 2018). These observations provide some initial assessment of transport and storage patterns for MPs, but as evidence is limited to three studies, little remains understood, thus further research is necessary to support initial observations. This can only be achieved by combined assessment of both sediment and water samples across variable spatio-temporal conditions such as sediment composition and seasonal flow patterns. Additionally, seasonal flow patterns may also provide information on the composition of MPs transported from the catchment to the rivers. For example, in Japanese rivers, concentrations increased with water level, which the authors attributed to greater release of MPs from non-point sources during rainfall events (Kataoka et al. 2019).

Other studies have also further expanded our understanding on the potential contribution of different sources of MPs to river by exploring the relationships between MP concentrations and basin characteristics, such as proximity to WWTPs (Baldwin et al. 2016; Leslie et al. 2017; Vermaire et al. 2017), land use (Baldwin et al. 2016; Ballent et al. 2016), population density (Baldwin et al. 2016; Horton et al. 2017), and industrial activities (Ballent et al. 2016). In general, MP concentrations tend to be higher downstream from WWTPs compared to upstream sites not receiving effluent discharges (Leslie et al. 2017; Vermaire et al. 2017; Shruti et al. 2019), supporting the notion that WWTPs are important conduits of point source MP contamination. The release of MPs from sewage treatment plants warrants further assessment, especially as extensive colonisation of MP pellets and fragments has been observed, and bacterial assemblages on the plastisphere were identified primarily as plastic degrading taxa and common human intestinal pathogens (McCormick et al. 2015; McCormick et al. 2017). Furthermore, positive associations have been observed between MP abundances and urban attributes. For example, higher MP pollution was present in areas with higher population densities and nearby industrial complexes for manufacturing of plastics products (Ballent et al. 2016; Shruti et al. 2019), textiles and clothing (Shruti et al. 2019) that can be sources of primary-type MPs and fibres. While diffuse sources are harder to quantify, it has been noted that predominance of fragments may be indicative of nonpoint pollution due to breakdown of parent materials, for example plastic waste discarded by visitors to nearby parks (Wen et al. 2018) and fishing activities in the river (Wang et al. 2017).

The relationship between MPs and various physical and chemical parameters of the rivers have also been discussed (Zhang et al. 2017; Nel et al. 2018; Rodrigues et al. 2018; Kataoka et al. 2019). For example, a positive association was observed between MP abundances and BOD in Japanese rivers, suggesting that sources and inflow of MPs to rivers may be similar to other pollutants, and that

systems with higher levels of pollution can be expected to have more MPs contamination (Kataoka et al. 2019). Furthermore, elemental analysis by SEM-EDS showed that MPs contained organic solvents and pharmaceuticals (Di and Wang 2018), as well as inherent and environment-derived metals on their surface (Wang et al. 2017a), also considered priority pollutants that can impair water quality. Lastly, some associations have been observed between MP concentrations in river waters and those in fauna. For example, ingested MPs as PE and nylon were found in the guts of fish in the Xiangxi River (Zhang et al. 2017). One study proposed the use of Chironomids as an indicator species of MP pollution in river systems as a positive correlation was established between MPs levels in these organisms and in sediments of the Bloukrans River in South Africa (Nel et al. 2018). The associations between MPs and aquatic fauna can serve to evaluate risk of exposure and provide insight in some pathways for removal and degradation of these contaminants by biofouling.

Although variability in MP concentrations is influenced by environmental conditions and basin characteristics, differences in method selection can also contribute to differences in results across various studies, making comparability challenging. For example, different sampling protocols can alter the lower limit of detection and the types of particles that are captured (Leslie et al. 2017). Sediment sampling is often achieved by collection of bulk sampling, although a UK study in the Rivers Irwell and Mersey used a cylinder resuspension apparatus (Hurley et al. 2018). This UK survey reported the highest concentrations so far, but it is not possible to assess from the information provided whether this was due to the specific catchment characteristics or may have been influenced by the sampling approach. For surface water sampling, most studies use stationary or trawl nets with mesh typically 300-335 µm (**Table A-2)**, but this tends to underestimate smaller MPs, which the literature reports are likely to be more abundant. A few studies have employed grab-sampling to allow for smaller mesh sizes to be used (**Table A-2**), although this usually means a trade-off for smaller sampling volumes. While studies using net sampling do not report sampling volumes, deployment times can range from a few minutes to an hour and authors report that this approach allows filtering of larger sampling volumes (e.g. 100,000 L, Vermaire et al. 2017). Conversely, 1 to >100 L have been obtained via grab sampling in a few surveys (**Table A-2**). Two studies explored the effect of different water sampling techniques on MP quantification (Dris et al. 2015; Vermaire et al. 2017). In the River Seine in Paris, nets with two mesh sizes (80 and 330 µm) were tested, with higher MP concentrations observed when the smaller mesh size was used (Dris et al. 2015). A different study compared MP concentrations obtained by grab versus manta-trawl sampling of surface waters, observing that manta-trawl estimates were systematically lower than those estimated by grab-sampling but it was uncertain if this reflected differences in sampling location or volumes (Vermaire et al. 2017). Furthermore, while both techniques showed a predominance of microfibres, net trawls had a greater number of beads and fragments (Vermaire et al. 2017).

To summarise, this chapter introduced and reviewed the current literature on MP contamination on freshwaters and wastewater treatment systems, the methods for their study, and finished with an expansion of the current knowledge specific to freshwater rivers generated since the study was initiated. While quantitatively, it may be difficult to compare studies due to differences in methodology and objectives, some collective messages can be identified from previous research. Microplastics are ubiquitous in the environment and have been detected in oceans, freshwaters, wastewaters and their biota worldwide. However, abundances across and within systems are highly

variable and the main drivers of spatial and temporal variations remain poorly understood. The limited information available for wastewater research revealed that MPs in incoming water are predominantly in the form of fibres and microbeads, because these are released into household sewage from washing of synthetic garments and use of personal care products. Even though WWTPs are not designed to purposely remove MPs, current treatment processes seem to exhibit high removal efficiencies, usually higher than 90% although this is variable (see Section 5.1). While preliminary and primary treatment stages are often reported to remove most of the incoming MPs, understanding on the efficiency of secondary treatment by conventional activated sludge and advanced treatment technologies in removing MPs remains incomplete and requires further insight. Despite the high removal efficiencies achieved by current treatment technologies, treated effluent may discharge millions of MPs into the recipient waters daily.

The role of WWTPs as pathways for MPs to enter the aquatic environment is supported by findings of increased MP concentrations in rivers downstream of WWTP discharge compared to upstream sites. However, spatial patterns of MP contamination in rivers have also demonstrated that WWTPs are not the only route of entry of MPs to rivers as other point and diffuse sources in the catchment can also introduce anthropogenic contamination. In general, MPs concentration in freshwater rivers tends to be higher near urban, industrial, and densely populated areas, with the River Mersey in the UK (Hurley et al. 2018) and the Yangtze River in China (Di and Wang 2018) presumed to be the most polluted river systems considered so far. Findings from more recent river studies suggest that once in the river system, MPs are more likely to be retained in sediment during periods of low flow as a reduced water velocity promotes sedimentation processes, while high flow events lead to resuspension and flushing of MPs from these temporary sediment sinks (Hurley et al. 2018).

Indeed the MP literature has evolved at a fast pace and new publications became available during the time this research was carried out. Despite this rapid growth in MPs research, the marine literature is growing five times faster than freshwater studies (Blettler et al. 2018), with even fewer publications for wastewater treatment systems, and most fresh- and wastewater studies have been conducted in developed countries. Additionally, some of the observed variability across studies may be artificial due to the differences in study aims and methodology, with different studies employing different sampling schemes and combinations of visual and chemical techniques to quantify and characterise MPs. As a result, studies, especially those for freshwater rivers may appear disconnected and it is difficult to compare findings quantitatively across sites, limiting current understanding on MP pollution and risk. Thus, more spatiotemporal data points are needed to develop a comprehensive understanding of the patterns of abundance and distribution of MP contamination in rivers and WWTPs, and the main drivers of retention and release of MPs across different compartments.

2.9 Research hypotheses

Based on the above analysis of the literature, the following hypotheses were formulated to support the general and chapter-specific objectives:

General hypotheses, corresponding to core objectives 1, 2, and 3, respectively:

1) MPs will be present in urban freshwater rivers and a WWTP in different shapes, sizes and polymer composition
2) Wastewater treatment will remove a portion of MPs, but not all
3) Spatial distribution of MPs in rivers will reflect the influence of point and diffuse sources of pollution and changes in hydrodynamics

Hypotheses under **Chapter 4**

1) MPs will be present in river sediment
2) Chemical analysis will improve MP characterisation

Hypotheses under **Chapter 5**

1) MPs will be present in incoming wastewater, especially microbeads released from personal care products and fibres from washing machine effluent
2) MPs concentration will decrease after each treatment stage, but some discharge may still occur

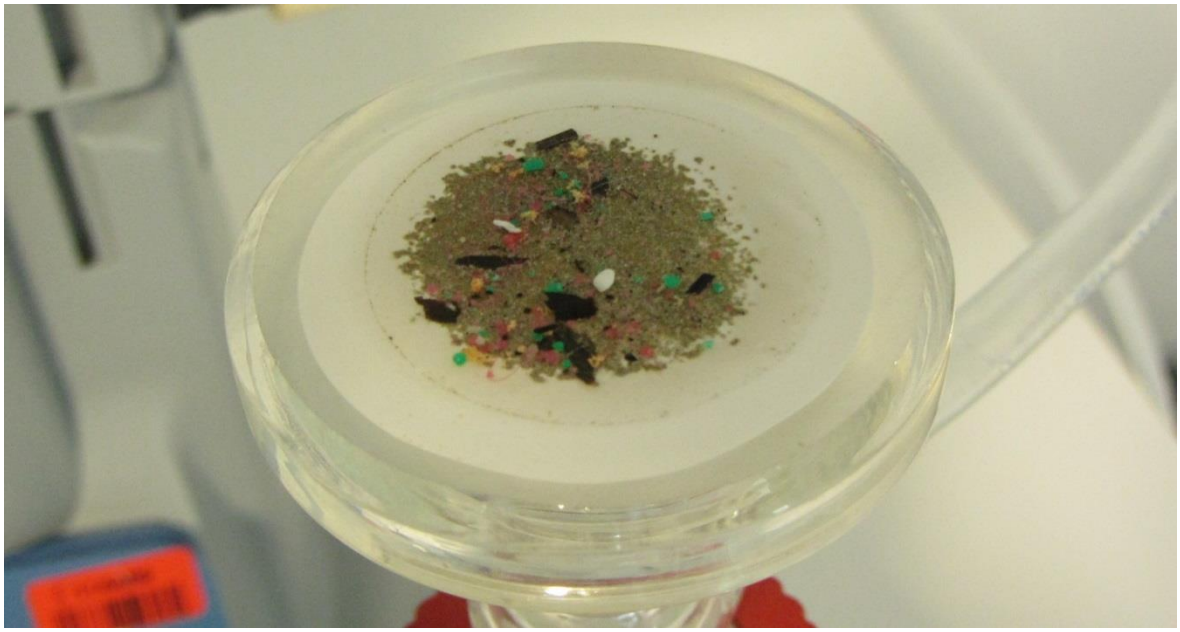
Hypotheses under **Chapter 6**

1) MPs will be present in river sediment and water
2) MPs will be higher downstream from WWTP compared to upstream sites
3) Retention of MPs by river will be reflected in increase in sediment MP concentration and decrease in water MP concentration, with decreased flows. Conversely, transport of MPs by river will be reflected in increase in water MP concentration and decrease in sediment MP concentration, with increased flows.

These general and chapter-specific hypotheses are discussed summarily in **Chapter 7** in conjunction with the core objectives of this research.

The next chapter introduces the selected study sites and general protocols followed for extraction and characterisation of MPs throughout the research, and expands the theoretical framework for the two main chemical characterisation techniques used here, with some reiteration of the collective messages for freshwater literature.

3 Overview of Methods



Abstract

Standardised protocols for MPs research are not available, thus the methodological approach employed in this study was designed broadly from methods summarised previously (Hidalgo-Ruz et al. 2012; Masura et al. 2012. MERI 2015; Qiu et al. 2016) and briefly discussed in the literature review. However, protocols vary greatly across studies and often enough details are not provided, thus the experimental design also draws from standard protocols for collection and analysis of soil and water samples based on the student's research background in soil and water sciences. As different catchment conditions may not allow for direct implementation of protocols employed in previous surveys, once the generalised protocol was established, this was calibrated and adapted for the different compartments and according to the specific objectives of each component of this study (**Table 3-1**). This chapter provides a general description of protocols while further details are provided in the following empirical chapters (sections 4.2, 5.2, and 6.3), for ease of reading.

3.1 Study sites

The main study site was the upper River Clyde catchment in Glasgow, Scotland (**Figure 3-1**). Sampling points were located across three main systems: the River Kelvin, Daldowie WWTP, and the River Clyde. A brief overview of each sub-catchment follows in the same order as the data chapters.

3.1.1 River Kelvin

The River Kelvin is a freshwater river and a main tributary to the River Clyde (Matheson 2000). It has its source near Kelvinhead, from which the river flows west and south until its confluence with the River Clyde Estuary, draining through the counties of Stirlingshire, Dunbartonshire, and Lanarkshire in Scotland (**Figure B-1**; Matheson 2000). Along its course of 34 km, it receives waters

from various burns and three main tributaries, which are the Luggie and Glazert Waters near Kirkintilloch, and the Allander Water near Bearsden (Matheson 2000). From Kelvinhead to its confluence with the Allander, the river drains primarily west through rural land, but the convergence of the Allander Water steers the course of the Kelvin south towards the City of Glasgow (Matheson 2000). After reaching the northern boundary of the city, the River Kelvin continues initially through rural areas, then becoming an urban river at Maryhill and until its discharge into the River Clyde at Partick, in the west end of Glasgow (Matheson 2000).

Historically, the Kelvin has undergone several changes. Originally, a meandering river, the Kelvin was modified to prevent risk of flooding, but these man-made changes altered its original course (Moore et al. 2017). Furthermore, during the industrial revolution, various weirs and dams were built along the river, and it received substantial pollution as a number of industries utilised its waters for their operations while also discharging their effluent back into the channel (Matheson 2000). The impaired water quality of the River Kelvin killed its biota and contributed a heavy pollution load to the River Clyde (Moore et al. 2017). However, in recent years, efforts including cessation of sewage discharges to the channel, were successful in improving the water quality of the River Kelvin and returning salmon and other fish and bird populations (Matheson 2000; SEPA 2006). As a river system exposed to various anthropogenic stresses, this catchment can provide a good setting for assessing multiple diffuse and historical sources of MPs.

Sampling in the River Kelvin for this study included sediment collection from one site before its confluence with the River Clyde Estuary. Further details on the collection site and sampling for the River Kelvin experiment are provided in section 4.2.1.

Table 3-1 Summary of methods employed for sampling, extraction, and characterisation of MPs from sediment, wastewater and water samples from the River Kelvin, Daldowie WWTP, and the River Clyde. **SE**, sampling event

Study Site	Study Period	SE	Type of Sample	Sampling Method	Sample Points	Extraction Method	Size range	Filter pore size (µm)	Identification Method	Quality Controls	Processing Time per SE
River Kelvin	12/2015 – 02/2016	2	Sediment	Bulk sampling of sediment using spade	1	NaCl density separation, filtration	>2.8, 2.8-1.0, 1.0-0.5, 0.5-0.3, 0.3-0.1, 0.1-0.7, 0.7-0.18, 0.18-0.09. 0.09-0.06, <0.06	11	Light microscopy SEM-EDS	Blanks, recovery tests	4 weeks
Daldowie WWTP	05/2017 – 02/2018	5	Wastewater	Grab sampling of wastewater with metal bucket	8	30% H_2O_2, filtration	2.8-1.2	1.2	Light microscopy SEM-EDS FTIR-ATR	Blanks, fragmentation tests, sample spiking	8 weeks
River Clyde	08-11/ 2018	3	Sediment	Bulk sampling of sediment using spade	5	NaCl density separation, filtration	2.8-1.0, 1.0-0.3, 0.3-0.18, 0.18-0.06, <0.06	1.2	Light microscopy FTIR-ATR	Blanks, recover tests, sample spiking	1 week
River Clyde	02-03/ 2019	2	Surface water	(1) Grab sampling of water with bucket + on-site filtration (63 µm); (2) Composite 5-30 min sampling using plankton net (63 µm)	2	Filtration only	2.8-0.63	1.2	Light microscopy FTIR-ATR	Blanks, sample spiking	1 week

Figure 3-1 Sampling points located in the upper River Clyde catchment in Glasgow, Scotland, relative to the Firth of Clyde estuary: (1) River Kelvin near confluence with the River Clyde; (2) Daldowie tertiary WWTP; (3-5) River Clyde downstream of the WWTP; (6-7) River Clyde upstream of the WWTP. ArcGIS online basemap copyright © Esri. All rights reserved.

3.1.2 Daldowie tertiary WWTP

Selection of the wastewater treatment facility was agreed jointly with Scottish Water and SEPA. During the selection process, eight sewage treatment works in the Clyde catchment operated by Scottish Water were short-listed and evaluated according to specific criteria (**Table B-1**). Daldowie tertiary WWTP was selected as the study site as it met most of the desired conditions as follows:

- It discharges into the upper River Clyde, providing a freshwater system draining through an urban catchment and is in close proximity to the marine environment.
- It is a relatively large facility operated by Scottish Water with 184,500 p.e. and receives a mix of trade and domestic sewage, making it a good representative of the Scottish population.
- It uses an activated sludge system, reflecting current and future tendencies for Scottish Water as preference would be to use activated sludge over percolating filters for upgrades.
- It has tertiary treatment, offering the opportunity to assess the effect of this additional treatment step.

Details on the WWTP site and the sampling scheme for this experiment are provided in section 5.2.1, although the site was not named in the published paper, upon request by Scottish Water.

Additionally, two 1-L activated sludge samples were obtained from Shieldhall WWTP, a large sewage treatment plant in Scotland serving a population of 500,000 or more. The treatment plant employs pre-treatment of raw sewage by screening and grit removal, then primary settling, and finally secondary treatment by a conventional activated sludge process (Rudd 2014). Shieldhall WWTP (55.866927, -4.341304) was built in 1910, and was upgraded starting in 1975 as part of the Bundle 25A project, with phase 1 aimed at improvements to the preliminary and primary treatment equipment, and phase 2 consisting of modifications to the secondary treatment machinery (Rudd 2014). Activated sludge samples are collected regularly by operations staff at the facility and then examined by microscopy for monitoring the health of the microbial community. By lowering a metal container into the sludge stream, two 1-L grab samples from their routine sampling were collected and provided by Scottish Water staff in April 2017. These samples were used for calibration purposes initially in preparation for the Daldowie work; however, the extended processing times for wastewater samples did not allow for sludge sampling to be carried out at Daldowie. Thus the two Shielhdall samples were used as proxy to assess the possible occurrence of MPs in biosolid fractions as both plants are located in the same catchment, serve a similar population demographic, and discharge into the River Clyde.

3.1.3 River Clyde

The River Clyde is Scotland's second longest river after the River Tay, with an estimated length of 170 km (Pollard 1998). From its source at the confluence of the Daer and Potrail Waters in the Lowther Hills (Pollard 1998), it flows north and northwest through Glasgow and into the Firth of Clyde estuary in Dumbarton and Greenock (**Figure B-2**). The river receives water from numerous tributaries, including South and North Calder Waters, the River Kelvin and the White Cart (Pollard 1998) and discharges from several WWTPs, such as Dalmarnock, Daldowie, and Shieldhall.

Historically, the Clyde played an important role for commerce and industry in Glasgow and, similar to the River Kelvin, saw negative impacts of pollution and man-made modifications during the industrial revolution (McLeod and Gilroy 1996). Textile and mining industries, for example, were established along the Clyde bank, leading to a boom in international shipping trade and a deepening of the river channel to allow docking of larger ships (McLeod and Gilroy 1996; Pollard 1998). Water from the Falls of Clyde were diverted for hydroelectric power generation of mills in New Lanark (Pollard 1998). As a result of urbanisation and industrialisation, the River Clyde was considered one of Europe's most polluted rivers until the 1960s, and its poor water quality made it unsuitable for aquatic fauna to survive (Pollard 1998). Efforts started around 1965 to remove heavy industrial and mining activities and regenerate the River Clyde, thus improving water quality and promoting the return of various fish populations (McLeod and Gilroy 1996; Pollard 1998). Currently, the River Clyde catchment provides an area for transport and numerous recreational activities, such as water sports, sailing, and fishing.

The River Clyde is classified into upper and lower sub-catchments by the Clyde River Foundation, using the Falls of Clyde at New Lanark as their boundary. Work for this study was conducted in the upper River Clyde catchment. Spatial sampling on the River Clyde was conducted across up- and downstream sites from Daldowie WWTP sewage discharge. Sampling points were selected based on their location relative to the discharge pipe, as well as their proximity to other potential sources of anthropogenic waste, including roads and pedestrian paths, residential and industrial areas, combined sewer overflows (CSOs), and SEPA's Scottish Pollutant Release Inventory (SPRI) points. The SPRI is a database of annual pollutant emissions and off-site waste transfer from regulated industrial sites in Scotland (https://www.sepa.org.uk/environment/environmental-data/spri/). The pre-selected sampling points were then visited to assess accessibility to the river bank for a final selection of five collection sites (**Figure 3-1**). Similar to its tributary, the River Kelvin, this system was and is still exposed to various anthropogenic pressures, so can provide a good representation for point and non-point sources of MPs. Furthermore, its proximity to the marine environment can provide insight into the delivery of marine MPs from land-based sources.

Sampling in the River Clyde consisted of sediment collection from five sites and water collection from two sites. Further details are provided in section 6.3.1 on the sampling scheme.

3.2 Extraction of MPs

In general, extraction of MPs from the sampling matrices was achieved by density separation of MPs from sediments with a saturated NaCl solution with a density of ~1.2 g cm^{-3}, and by digestion of labile organics in wastewater and sludge with 30% H_2O_2 (**Table 3-1; Figure C-1**). River water samples were untreated. All samples were filtered by gravity or under vacuum to capture MPs for subsequent identification (**Figure C-1**). Methods for MP isolation from sediment and liquid wastewater fractions are detailed in sections 4.2 and 5.2, respectively. The general protocols described in Chapters 4 and 5 were used as base for sampling and extraction of MPs from sediment and water samples in the River Clyde, thus the modified protocols are described in the respective chapter (section 6.3).

3.3 Contamination controls

Background contamination occurs frequently in MP studies, especially for fibres that can be airborne and deposited during sampling or processing. Furthermore, fragments could be produced by the mechanical breakdown of plastic equipment used during the sampling or extraction process. Precautions were taken in this study to minimise potential contamination from atmospheric fallout, for example by using adequate protective equipment (e.g. a white lab coat, nitrile gloves, procedure masks), reducing the use of plastic materials, and keeping samples covered with aluminium foil when not being processed. However, eliminating the use of plastic equipment was not always possible, and controlling sources of background contamination in the lab is difficult as fibre deposition has been observed even under sterile conditions (Wesch et al. 2017). Therefore, quality control tests were performed throughout all experiments to account for this potential for analytical bias (**Table 3-1**). Atmospheric controls were created by placing open containers filled with DI water on benches during extraction runs in order to capture airborne contamination from the lab environment. Contamination of MPs from sampling equipment was assessed by replicating the sampling process using DI water to create procedural blanks or by visual and chemical characterisation of fibres from sampling ropes. Additionally, method validation tests were conducted with the use of primary (**Figure C-2**) and secondary MP standards **(Figure C-3**). These standards were used to spike field samples and blank controls to estimate recovery rates and the potential for generation of further MPs by mechanical breakdown of materials from the extraction process. These tests are described in detail in Chapters 4-6.

3.4 Characterisation

3.4.1 Light microscopy

For all samples, light microscopy was the first stage of identification and enumeration, and was required since the subsequent characterisation step depended on the ability to first identify pieces and then manually transfer them for instrumental analysis. Visual sorting was always performed with a Leica MX_{75} microscope with magnification ranging between 10x and 32x. In general, at this stage MPs were classified into primary or secondary categories. Primary MPs included pellets, referring to spherical or cylindrical MPs resembling those used in personal care products (e.g. facial scrubs and toothpaste) and pre-production pellets. Secondary MPs were subdivided into fibres, fragments, and films. Fibres included elongated pieces of various lengths that may be released during washing or daily use of clothes, as well as those resembling fishing lines and rope fragments. Fragments and films consisted of any other 3-D or 2-D particles, respectively, that appeared to break off from larger pieces and thus had irregular shapes and edges. Colour was also observed in this study, although pieces were simply categorised as pale or coloured. Here, pale MPs referred to transparent, white and cream particles, while coloured MPs, as the term may imply, included all other colours (e.g. red, blue, black, yellow, green) (**Figure 3-2**).

Figure 3-2 Example of a pale bead (top left) and film (top right), and coloured fibres (bottom left) and fragment (bottom right) observed in samples obtained from different sites in this research

While visual inspection is consistently used across MPs research studies, the categories used in this or previous studies to describe these materials are not standardised. As MPs are highly diverse, maintaining consistency during visual identification can be difficult, but some generalised rules or criteria have been proposed to improve reliability of this step (Hidalgo Ruz et al. 2012; MERI 2015). For example, tweezers or other probes can be used to test the pieces since plastics are flexible and will not break when touched, but may melt or curl when prodded with a hot needle (MERI 2015). Furthermore, plastics will lack cellular or organic structures (Hidalgo-Ruz et al. 2012), however some exceptions may occur where biofouling may leave some organic residues, but for MPs these will only be present on a portion of their surface (MERI 2015). Single MP particles will often exhibit clear and homogeneous colours and MP fibres will have a consistent thickness throughout their length (Hidalgo Ruz et al. 2012), although there may some variations to these rules (MERI 2015) and thus each particle should be assessed in as much detail as possible. As a general rule, visual identification may not be reliable for smaller pieces but the cut off limit will vary by case and depends on the observer. For this research, visual identification was possible to ~60 µm, based on the size fractionation data. However, where there is uncertainty if a particle is plastic or not, it is best to not include in the final counts as this provides more conservative estimates (MERI 2015). While visual inspection is currently an obligatory step and provides an initial assessment of MPs in environmental samples, this approach will not give certainty that a piece is made of synthetic polymers. Confirmation of MP particles can only be achieved by chemical characterisation and thus this was

the second step in the MP characterisation process for this study. Here, SEM-EDS and FTIR-ATR were the two chemical techniques used to refine the accuracy of visual counts.

3.4.2 SEM-EDS

The first technique used for chemical characterisation in this study was SEM-EDS. This technique has been cited as a successful approach by a few studies in discriminating plastics from other confounding materials (Zbyszewski and Corcoran 2011; Eriksen et al. 2013), and can provide important information on various physical and chemical properties of pieces including size, shape, crystallography and composition (Goldstein et al. 2018).

For SEM-EDS analysis of MPs, the specimen is mounted on a C-adhesive and placed in the chamber area (**Figure C-4**), then scanned by focussing an electron beam with high energy (e.g. 20-30 keV) on the surface of the sample (Goldstein et al. 2018). The sample-electron interaction produces different types of signals including BSE and secondary electron (SE) images (Goldstein et al. 2018; Nanoscience Instruments 2019). Backscattered electrons are beam electrons that are scattered after elastic collision with the atoms in the sample. These electrons emerge out of the sample with their energy almost intact and are captured by the detector to produce the BSE image (Goldstein et al. 2018). As BSEs are proportional to atomic numbers, this provides information to distinguish different materials as elements with different atomic numbers will produce different contrast in the image (Goldstein et al. 2018). In the case of plastics, the specimen would show little to no contrast against the C-adhesive background allowing for a quick screening of potential MPs (**Figure 3-3**). Secondary electrons are those ejected from the sample surface due to inelastic scattering from the electron beam-sample interactions and have low kinetic energy (Goldstein et al. 2018). The SE image can be used to gather information on the structure of the sample surface to assess degradation and erosion of particles as these processes can leave visible signals on the plastic surfaces (Hidalgo Ruz et al. 2012). In addition to BSE and SE images, the interactions between the electron beam and the sample can also generate an x-ray spectrum that can be analysed by EDS to determine the elemental constituents of the sample and their relative proportions, where each peak is assigned to an element (Goldstein et al. 2018). For MP analysis, the EDS spectrum would show a strong C peak (**Figure 3-3**) as plastics are C-based materials. Here, the BSE image and EDS spectrum were used to assess composition.

Scanning electron microscopy offers an advantage over light microscopy as it can offer higher resolution usually between 1-20 nm (Nanoscience Instruments 2019) thus can be a powerful tool for initial sorting and can aid in identification of smaller particles. In this research, SEM-EDS was used in the River Kelvin experiment with the purpose of screening of the sample to eliminate pieces with non-plastic signals, like metal or glass pellets and fragments that may resemble their plastic counterparts. This technique was useful to become familiarised with the appearance and morphology of MPs at the beginning of the project and to refine techniques to improve their identification. While SEM-EDS was commonly mentioned in earlier MP studies, its popularity seems to be decreasing in the recent literature while vibrational spectroscopy techniques become more widely used, especially infrared spectroscopy.

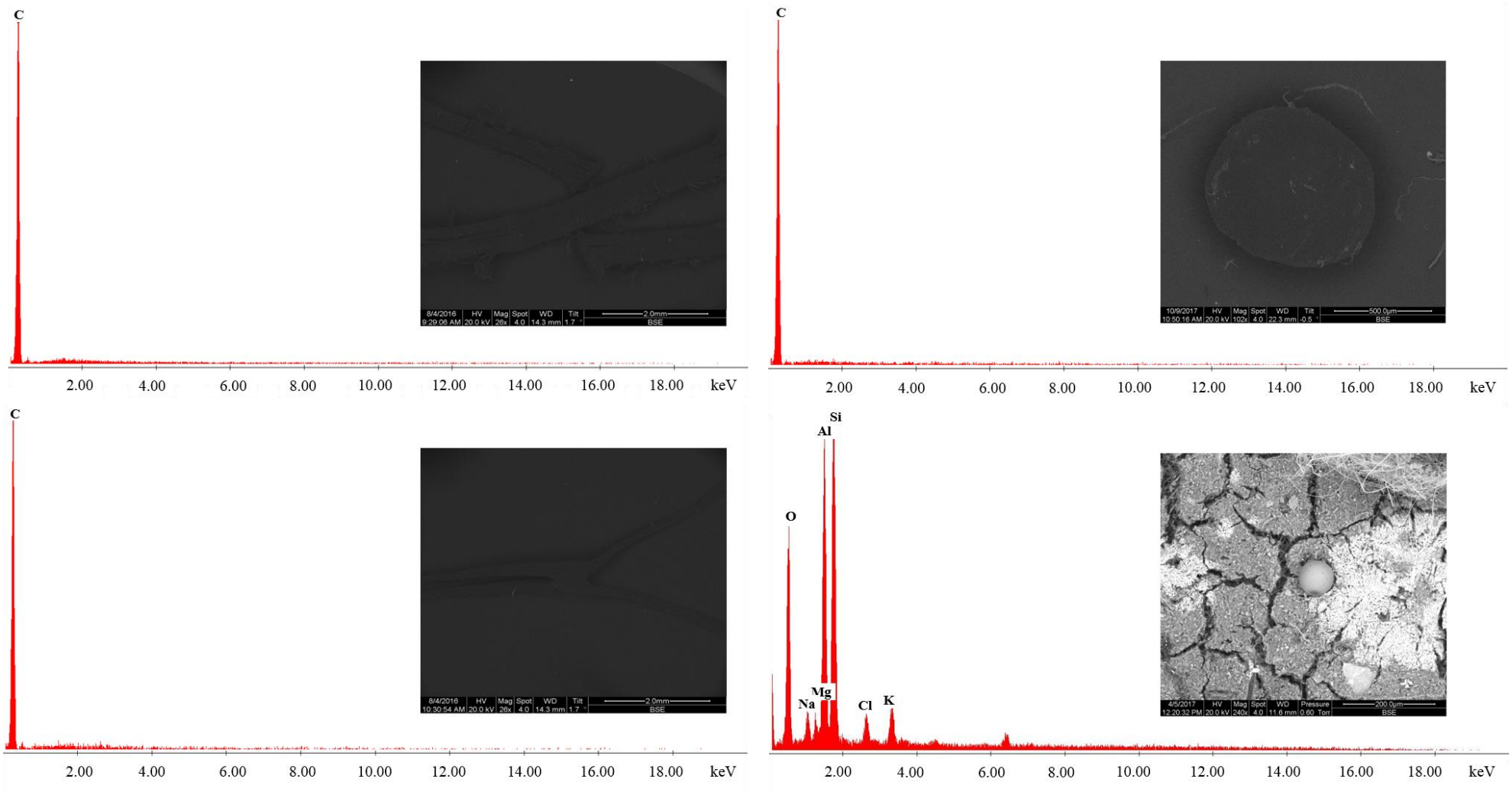


Figure 3-3 SEM-EDS BSE images and elemental spectra for PP (top left) and PP (bottom left) fibre standards, PE bead standard (top right), and non-plastic silica bead (bottom right). BSE image shows contrast among materials with different chemical composition: plastics will display little to no contrast against carbon background.

3.4.3 Vibrational spectroscopy by FTIR-ATR

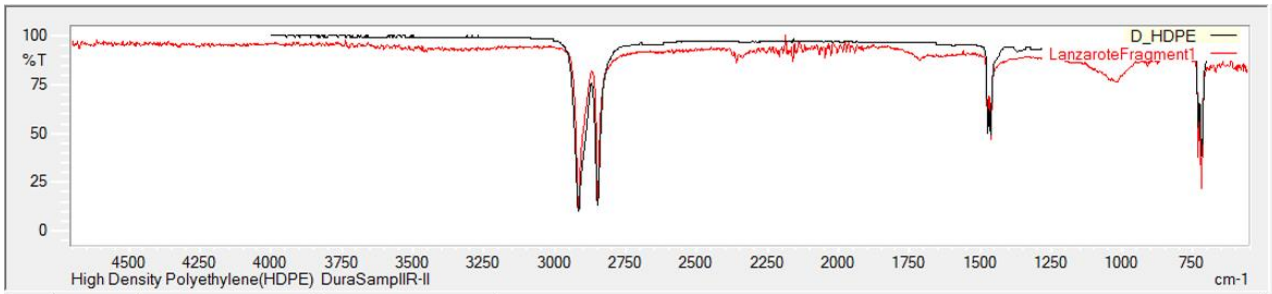
Infrared spectroscopy is a type of vibrational spectroscopy technique used to identify unknown compounds, thus in MPs research it is employed for characterisation of synthetic plastics and their separation from other C-based materials, which is not possible by light or electron microscopy. The information gathered from this approach is needed for improving accuracy of results and can be used for tracing compounds back to their sources of origin, which is crucial for regulation of MP inputs. This spectroscopy method relies on measuring the vibrational energy of chemical bonds in a compound, hence the term vibrational spectroscopy (Coates 1996). In brief, the infrared radiation excites the bonds in molecules to vibrate at certain wavelengths that correspond to the unique molecular structure of the material (Coates 1996). While infrared spectroscopy can be employed in different modes such as transmission, reflectance and ATR (ThermoFisher Scientific 2018), in this research, FTIR-ATR tools were used for characterisation of MPs in the WWTP and Clyde experiments, as this equipment was available at the University facilities and provided a relatively rapid means to identify material type compared to alternative modes. A Raman spectrometer that is also employed for MPs research was not selected due to limitations of processing times, as Raman analysis time is higher than FTIR.

The FTIR-ATR technique is based on identification by sample contact, meaning that the spectral data are collected from a single point of contact at a time, as opposed to other mapping techniques like focal plane array (FPA) that produce resolution spectra (Primpke et al. 2017). Characterisation is achieved in the mid-infrared spectrum (400-4000 cm^{-1}), as most materials will fall within this range (Coates 1996). In FTIR-ATR analysis, the unknown specimen is transferred manually to the equipment and placed over a transparent crystal with a high refractive index (e.g. diamond, germanium) in the ATR accessory (**Figure C-5**). In MPs research, a diamond crystal with a refractive index of 2.4 is often used, the same as in this study, although a higher refraction may be necessary for dark particles like black rubber (ThermoFisher Scientific 2018). Once the piece is in place, the pressure clamp is lowered to press the sample for contact against the crystal, the infrared beam passes through the crystal, comes in contact with the sample, and is reflected back through to generate the spectral fingerprint (ThermoFisher Scientific 2018). The spectrum generated can be entered into a software that checks the spectrum against a library or database of known polymers and other materials to confirm the identity of the compound. While studies may differ in the databases used in the spectrum search, a number of commercial libraries are available. The spectrum search will often provide more than one result along with a score that corresponds to the probability of the match (**Figure 3-4**). Typically, scores of 700 or more are considered "good", but in MPs research, as plastics may be degraded, contain other substances, and there are no set guidelines, it is ultimately the researcher that makes the final judgment on what is considered the best match. For this research, materials were identified by comparing the unknown spectra to those in the Shimadzu LabSolutions IR libraries, which contain approximately 12,000 reference spectra. For each particle, the top three automated matches were assessed visually for accuracy and a minimum score of 700 (from a maximum if 1000) was deemed acceptable, below which particles were classified as unknown. It was considered that there is likely to be an error involved with the manual interpretation as this may be subjective and lead to different findings, thus this selected approach would provide consistency and robustness.

Vibrational spectroscopy approaches like FTIR-ATR offer powerful tools for confirmation of MPs occurrence and distribution, but analysing all particles is not feasible because, depending on the number of particles to be analysed, this technique can be time-consuming and costly. Here, at least 5% of the pieces identified visually were selected for this analysis. The subsample included representative pieces from all categories, and while proportionally equal amounts were pre-selected for subsampling within each categories, often pieces would be too small to collect and would get lost during transfer, thus could not be processed. In particular, pellets were difficult to measure as these were on the lower end of the size range possible for FTIR-ATR and were easily flicked or lost during transfer due to their smooth surfaces and static. As this technique depends on manual transfer of pieces and contact between the specimen and the crystal, analysis was limited to pieces >300 µm. Other IR imaging modes like FPA that produce resolution spectra and can be modified to scan an area containing MPs may enable this technique to analyse pieces down to 10 µm (ThermoFisher Scientific 2018), but these were not available for this study.

The total of confirmed plastics within each category was converted into a percentage and this percentage was used to correct the rest of the blank-correct visual data (FTIR-corrected data), to account for the error in counting non-plastics like cellulose as MPs during visual sorting.

To summarise, the study sites and methodological framework were broadly described in this chapter. Further details for the techniques presented here are provided in the following empirical chapters (4-6). The methods were calibrated for each site according to the specific objectives of each component of the research, starting with the work conducted in freshwater river sediment presented described in the next chapter.



	Score	Library	Name	Comment
1	888	107 - ATR-Polymer2	D_HDPE	High Density Polyethylene(HDPE) DuraSamplIR-II
2	860	23 - ATR-Polymer2	D_PE	Polyethylene(PE) DuraSamplIR-II
3	852	146 - ATR-Polymer2	D_Polyethylene_Oxidized	Polyethylene, Oxidized DuraSamplIR-II
4	838	4 - ATR-Polymer2	D_EAA	Ethylene Acrylic Acid(EAA) DuraSamplIR-II
5	777	100 - ATR-Polymer2	D_Ethylene_EthylAcrylate	Ethylene/Ethyl Acrylate Copolymer(Ethyl Acrylate content 18%) DuraSamplIR-II
6	776	123 - ATR-Polymer2	D_PE_PP	Ethylene/Propylene Copolymer(Ethylene content 60%) DuraSamplIR-II
7	770	221 - IRs ATR Reagent2	221	Cetyl Alcohol CH3(CH2)14CH2OH
8	722	220 - IRs ATR Reagent2	220	1-Tetradecanol CH3(CH2)13OH
9	715	36 - ATR-Organic2	D_StearateMg	Mg Stearate DuraSamplIR
10	711	49 - IRs ATR Reagent2	49	Decalin C10H18

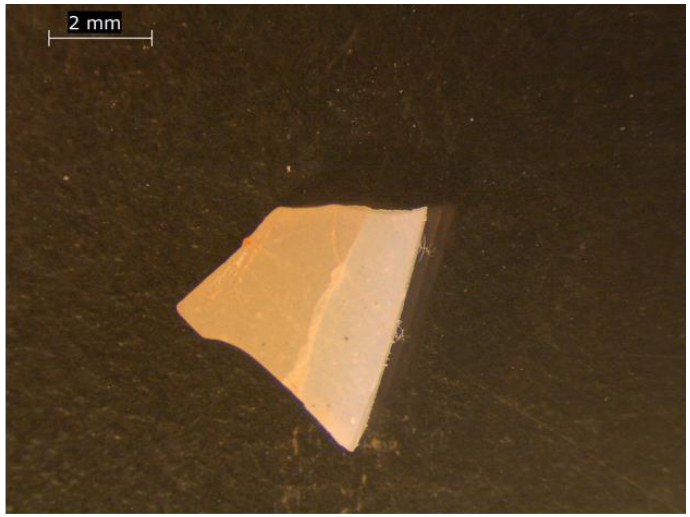


Figure 3-4 Example of FTIR-ATR output for a high density PE fragment (score of 888). Sample was analysed using a diamond crystal and 40 scans using a Shimadzu IRAffinity-1S FTIR. The material was identified by comparing the unknown spectra to those in the Shimadzu LabSolutions IR libraries, which contain approximately 12,000 reference spectra. The top three automated matches were assessed visually for accuracy and a minimum score of 700 (maximum 1000) was deemed acceptable, below which a particle would be considered of unknown origin.

4 Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK

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RESEARCH ARTICLE

Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK

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Abstract
Understanding of the sources, fate, and impact of microplastics (MPs, < 5 mm) remains limited, particularly in freshwater environments, while limited comparability across available surveys hinders adequate monitoring and risk assessment of these contaminants. Here, the distribution of microscopic debris in an urban river close to the marine environment in the West of Scotland was investigated to assess concentration and distribution of primary and secondary MPs. Also, the efficiency of light and scanning electron microscopy with energy-dispersive spectroscopy (SEM-EDS) was evaluated for characterisation and quantification of MPs sized 2.8 mm–11 μm. Bank sediment samples were collected twice from the River Kelvin in Glasgow and were size-fractionated and processed for extraction of MPs by density separation. Sample MPs spiking and use of procedural blanks allowed the influence of processing on field data quality to be considered. Total abundances were 161–432 MPs kg^{-1} dry sediment, with fibres as the dominant type, comprising > 88% of total counts. Nevertheless, fibres in blanks suggest potential contributions from atmospheric contamination. Moreover, fibres concentrated mainly in fractions < 0.09 mm suggesting that their fate may be influenced by drivers of fine sediment dynamics in rivers. While no primary MPs were observed, metallic and glass pellets were present in high abundances in settled material and could be easily misidentified by visual inspection, demonstrating that compositional analysis is needed to avoid analytical errors from MP misidentification and overestimation. SEM-EDS allowed for a quick screening of plastic vs non-plastic pellets and improved identification of smaller fragments, whereas more advanced techniques are needed for proper identification of fibres. This study is the first to report on MPs in freshwater rivers in Scotland and suggests that diffuse sources of pollution may be delivering secondary MPs to the river. Their sources, fate, and risk in these systems will thus warrant further attention.

Keywords Microplastic · Plastic pollution · Electron microscopy · Fibres · Freshwater · Sediment

Introduction

Plastic production and subsequent pollution are global environmental concerns. Global plastic generation has exhibited an upwards trend since the 1950s, reaching 335 million tonnes in 2016, a 10% increase from 2015 levels (Plastics Europe 2017). Moreover, an estimated 8300 million metric tonnes of plastic have been produced since 1950 to date, with

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Statement of authorship

RMB was responsible for the conception of the experimental design with feedback from supervisors, and performed all the experimental work and analysis and interpretation of data. RMB drafted the article for submission and was responsible for the incorporation of reviewers' comments and writing the response to comments. SW, VP and GCL contributed to the planning of the experiment and

editing of the manuscript. The content of the published material was revised with comments from two anonymous reviewers during the publication process.

Chapter introduction

The Clyde catchment represented an area for which there was no information on MP pollution in the literature, while other freshwater studies reported a wide range of pollution levels and limited information on the relative abundance of primary and secondary types of MPs. Therefore, the first part of the PhD project was planned to obtain an initial profile of the types of MPs that could be expected in the selected catchment. Furthermore, as no standard protocols are available and chemical characterisation techniques were in their early stages, another aim of this part of the project was to calibrate the sampling, extraction, and identification techniques for adequate measurement of the different types of MPs. This part of the research was published and the full paper now follows.

Citation

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Abstract

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4.1 Introduction

Plastic production and subsequent pollution are global environmental concerns. Global plastic generation has exhibited an upwards trend since the 1950s, reaching 335 million tonnes in 2016, a 10% increase from 2015 levels (Plastics Europe 2017). Moreover, an estimated 8300 million metric tonnes of plastic have been produced since 1950 to date, with approximately 6300 million metric tonnes of plastic waste created until 2015, of which only 9% was recycled (Geyer et al. 2017). Plastics are persistent materials, so when discarded as waste they can accumulate in landfills and the environment for a long time (Geyer et al. 2017) and pose a threat to biodiversity, ecosystems services and potentially human health (Eerkes-Medrano et al. 2015).

Arising from its aesthetic and environmental impacts, plastic contamination has received increasing attention from the public and scientific communities for several decades (Coe and Rogers 1997; Derraik 2002; Blair et al. 2017), especially larger, visible pieces. Of recent concern is microscopic plastic debris commonly referred to as microplastics (MPs), typically less than 5 mm in size (GESAMP 2015), although a formal definition and lower limit have not been established (Blair et al. 2017). They are divided, broadly, into primary or secondary types (GESAMP 2015), though these definitions are also not standardised. Primary MPs are produced intentionally and are typically small spherical pellets that can originate from their use in cosmetic and personal care products, as sand-blasting media, and pre-production pellets commonly known as "nurdles" (Storck and Kools 2015). Secondary MPs, such as fibres, fragments, and flakes are formed indirectly from the breakdown of larger plastic pieces. Sources of secondary MPs may be mismanaged plastic litter, release of fibres through everyday use and washing of synthetic textiles (Browne et al. 2011; Boucher and Friot 2017), and wear and tear of tyres, road markings and paints (Boucher and Friot 2017). Primary MPs have garnered the most media and public attention, prompting actions worldwide sometimes leading to country-wide bans on the use of microbeads (e.g., in the Netherlands, Canada, USA, United Kingdom, and New Zealand). Despite the greater focus on primary MPs, secondary types may be of increasing abundance, particularly fibres released into wastewater via washing machine effluent (Browne et al. 2011). Fragmented secondary MPs may increase in quantity over time, long after primary inputs are reduced since larger pieces may continue to degrade into smaller plastic particles. Currently, the contribution of different sources to overall MP loadings to the environment and the relative importance of primary and secondary types remains poorly understood (Duis and Coors 2016; GESAMP 2015).

Research focused on understanding the sources, distribution, fate, and impact of MP fractions in the environment is increasing rapidly (Blair et al. 2017; Horton et al. 2017), but knowledge of MP pollution in oceans compared to freshwater environments remains more advanced (Thompson et al. 2009; Wagner et al. 2014; Eerkes-Medrano et al. 2015). Coastal and beach surveys conducted between 1980 and 2001 worldwide, revealed that plastic waste can account for 50-90% of all marine litter and that MP materials have been accumulating rapidly in oceans and shorelines over the past few decades (Derraik 2002). More recently, interest in MPs in freshwater systems has been rising (Eerkes-Medrano et al. 2015) as these are known to be important transport vectors of land-based contaminants to coastlines and open sea environments. Widespread MP abundances have been observed in river and lake surveys of water and sediment samples collected from North American,

Asian, and European locations (Blair et al. 2017) with the highest concentrations in freshwaters to date observed in highly contaminated areas of Lake Taihu, China (Su et al. 2016) and in sediment of the River Tame (Hurley et al. 2018). Nevertheless, the role of fluvial waters as conduits of MPs to the marine environments from terrestrial sources has been largely unknown due to a lack of empirical data, although this is a rapidly growing field. Investigating the abundance and nature of MPs in rivers close to estuarine and marine environments, particularly in urban and industrialised catchments where MPs could be higher (Nizzetto et al. 2016; Hurley et al. 2018), can potentially further our understanding of this link.

Globally, there is high variability regarding MP abundances and distribution of primary and secondary types (Blair et al. 2017). This may be because MPs are highly diverse in shape, size, colour, and density, resulting in high variability in their distribution in space and time, even within localised environmental compartments. Thus, it is important to increase spatio-temporal coverage and generate further local and regional datasets to improve our understanding of this variability. Nevertheless, the diverse nature and small sizes of MPs render them difficult to measure and monitor (Hidalgo-Ruz et al. 2012; Tagg et al. 2015). Consequently, there is a lack of unified research methodology for isolation, identification and quantification of MPs both in oceans and freshwaters, reducing comparability among available surveys. Differences in sampling, density separation and sample digestion techniques, and visual assessment of MPs exist (Hidalgo-Ruz et al. 2012). Recently, analytical techniques have been employed more frequently to determine the chemical composition of the recovered pieces, a step that is important for discriminating MPs from other confounding materials that may be mistaken for plastics, for example cellulose fibres (Wesch et al. 2016). Current methodological limitations can lead to errors in characterisation and quantification of MPs from environmental samples, thus method validation of extraction and identification protocols should be routinely tested to understand where uncertainty can be introduced and improve the ability to characterise confidently.

This study sought to determine the prevalence and distribution (size, type and colour) of MPs in a site representing of sediment accumulation in the River Kelvin in the west end of Glasgow, Scotland, close to its discharge to the Clyde estuary. Combined physico-chemical characterisation approaches based on light microscopy and electron microscopy with energy dispersive spectroscopy (SEM-EDS) were used for identification and enumeration of microscopic debris from riverbank sediment. These were required to explore the viability of visual identification of MP and the need to draw on instrumental analysis in routine testing for source verification. This study contributes to generation of spatio-temporal datasets and understanding of what methods are needed for extraction and characterisation of MPs from freshwater environments globally.

4.2 Materials and methods

4.2.1 Site and sampling

The River Kelvin is a freshwater river in Glasgow, UK, rising near Kelvinhead in northern Glasgow and flowing southwest for approximately 34 km through woodland and marshland, and recreational and urban areas (Quadrat Scotland 2002). Near its source, the River Kelvin runs parallel to the Forth

and Clyde Canal then gradually increasing in volume, finally converging with the River Clyde Estuary in the west end of Glasgow (Quadrat Scotland 2002). Its close proximity to the marine environment makes it particularly suitable to evaluate the role of fluvial systems in the fate and transport of MPs from continental to oceanic waters. Bulk sediment samples from the surface to a depth of 8 and 10 cm, respectively, were collected with a spade in December 17, 2015 (sampling event 1, SE1) and February 15, 2016 (sampling event 2, SE2) from the River Kelvin bank (55° 52' 8.742", -4° 17' 19.0278", **Figure 4-1**). The sample site was selected to be representative of dense urban environments with nearby businesses, tourist attractions and residential areas, a road bridge, and a park. The site is located in a low-energy zone in the inner bend where the channel curves underneath the bridge, rendering it geomorphologically favourable for sediment deposition due to low stream energy and reduced velocity. Samples were collected in aluminium tins and wrapped in aluminium foil to avoid contamination by use of plastic containers, and transported to the laboratory five minutes away.

Figure 4-1 Location of the sample collection site in a river bend section in the River Kelvin in the west of Glasgow, Scotland, UK.

4.2.2 Sample processing

The methodological approach employed for sample processing broadly follows methods discussed in the literature (Hidalgo-Ruz et al. 2012; Blair et al. 2017). Throughout the process, a white lab coat (65% polyester, 35% cotton) and rubber gloves were used and care was taken to minimise sample contamination by avoiding the use of plastic materials where possible. As the laboratory is a busy environment and it is difficult to control contamination from nearby activities, blanks were used to account for background contamination.

First, samples were weighed in aluminium trays before and after oven-drying for at least 24 hours at 100°C, and mass of total solids (TS) in grams (g) was calculated as the weight of the dried samples. This temperature was selected as the average of methods proposed by Masura et al. (2015) and for standard determination of gravimetric soil moisture (Black 1965); and, as the threshold temperature for melting and decomposition of common thermoplastics (Klein 2011). Using an automatic shaker for a duration of 10 minutes, oven-dried samples were sieved into the following size classes: 2.8 mm, 2.0 mm, 1.4 mm, 1.0 mm, 0.71 mm, 0.5 mm, 0.355 mm, 0.25 mm, 0.18 mm, 0.125 mm, 0.09 mm, and 0.063 mm, producing 13 sub-samples for each sampling event. Size fractionation was employed to assess how different types of MPs are associated with different sediment grain sizes. Each size class fraction was weighed and stored in a glass bottle until further processing.

4.2.3 Extraction by density separation

After fractionation, density separation (DS) with a saturated NaCl solution (ρ ~1.2 g cm-3) was used to separate low-density MP pieces. Approximately 25 g (or entire volume if less than 25 g) of oven-dry sediment from each size fraction was mixed with 40-68 mL of salt solution to cover the sediment, manually shaken vigorously for 1 minute and left to settle overnight (~24 hours). After 24 hours, the supernatant was filtered through Whatman 11-µm cellulose filters to collect suspended debris. The filter paper was rinsed three times with deionised (DI) water to remove excess salt, then transferred to petri dishes to dry at room temperature (18-21ºC). During processing of SE1 samples, re-suspension of some settled sediment (i.e. those deposited after the 24 hour period) was observed during decanting. Thus, a second settling step was introduced for processing of SE2 samples in which the supernatant was transferred into a clean beaker before filtration, covered, and left to settle for two additional hours to allow for further settling of re-suspended solids and reduce their potential transfer to filters.

The DS extraction method was validated via recovery tests using river bank sediment collected from the same study site, spiked with different types of MP standards. Polyethylene (0.71-0.85 mm diameter, ρ =0.96 g cm^{-3}), polypropylene (2.45 mm diameter, ρ =0.866 g cm^{-3}), and polystyrene (4.4 mm diameter, ρ =1.048 g cm^{-3}) microbeads purchased from Cospheric LLC (Santa Barbara, California) were used to mimic primary MPs. Nylon toothbrush bristles and rope fragments, polypropylene cleaning brush bristles, and polyethylene mesh fruit packaging fragments produced in the lab were used to mimic fibrous secondary MPs. Briefly, approximately 20 g of oven-dried sediment were spiked with 10 beads or 15 fibre-like fragments, in triplicates for each polymer type,

thoroughly mixed, and processed the same way as field samples. Recovery efficiencies were calculated as [number of pieces extracted/number of pieces spiked] * 100).

Procedural blanks consisting of NaCl solution were produced with every filtration sequence to account for background contamination.

4.2.4 Identification and quantification

First, a stereo microscope was used to identify MPs based on physical appearance. Here, samples different from sediment grains (i.e. more rounded, pitted, fibre-like, coloured or transparent) were identified and counted, and pieces in sizes ranging <2.8 mm to 0.7 mm were picked out with metal tweezers into glass vials and photographed with a Leica MC120 HD camera connected to a Leica MX75 microscope with magnification between 10x and 32x, depending on the size of the particle. Pieces smaller than 0.7 mm were not extracted this way as they were too small to manipulate and could be lost during manual transfer; these fractions were counted and saved on the filter paper until further instrumental analysis. Settled solids were also inspected under light microscopy to detect presence of high-density polymers (ρ>1.2 g cm^{-3}).

Representative aliquots of suspected MPs from each category and size fraction were examined using a FEI Quanta 200F scanning electron microscope (SEM) coupled with energy dispersive spectroscopy (EDS), enabling determination of elemental composition. The aliquot was selected from the SE1 samples and comprised suspended and settled pieces. Briefly, samples were prepared by placing individual pieces >0.7 mm on double-sided adhesive carbon discs (9-mm diameter), mounted on 9-mm specimen stubs and imaged by SEM-EDS operating at an accelerating voltage of 20 keV in the secondary electron and backscattered mode. Suspended pieces <0.7 mm that could not be separated manually with tweezers were transferred onto the SEM stub by “pressing” the C adhesive over the filter paper and using a light microscope to verify that the target piece was successfully transferred onto the stub. If it was not possible to transfer a piece after multiple tries, a square of filter paper was cut around it and placed on the stub. The compositional data were used to discriminate plastics from non-polymers since the plastics are carbon-based and other materials are expected to be non-organic. Electron microscopy assessment of the aliquot was used to refine the approach to the visual identification of MPs for the remaining samples under light microscopy.

The sum of pieces counted in all size fractions was used to quantify MP abundance for each sampling event by visual characterisation under light microscopy (stage 1) followed by chemical characterisation by SEM-EDS analysis (stage 2) to compare visual and chemical assignation of MPs. Abundances were calculated as [total number of suspected MPs/mass of TS] and expressed in items per kg of dry sediment.

4.3 Results and discussion

4.3.1 Method validation tests and blanks

Recovery rates for MP microbead standards were 100 % for all polymer types, sizes, and densities (**Figure 4-2**) while average recovery rates for fibre-like secondary MPs were lower than for primary MPs, ranging from 49+10.2 to 58+7.7 % for mesh packaging fragments and nylon rope pieces, respectively (**Figure 4-2**). Lower recovery rates for fibrous MPs may be attributed to a tendency to cluster together and adhere to the inorganic matrix and walls of the container, and may present a challenge for separation and thus accurate quantification of this type of MP.

Fibres were the only type of materials observed in procedural blanks. Fibre content in blanks were similar to those observed in other studies (Dris et al. 2015; Horton et al. 2017; Hurley et al. 2018). Only a handful of freshwater studies have included use of blanks as verification, but when reported they were considered negligible compared to those observed in field samples (Dris et al. 2015; Horton et al. 2017) or determined to be non-plastic (Hurley et al. 2018). Thus, the field data were not blank corrected in this study. Nevertheless, their occurrence in blank controls suggests background contamination, meaning that the field samples may contain a non-river contribution of fibres that could result in overestimation. Conversely, their lower recovery rates could result in an underestimation in both the sample and the blank. As fibres seem to be a predominant MP category in this and many studies, more blank and standard control tests are needed to reduce these uncertainties and improve confidence in results.

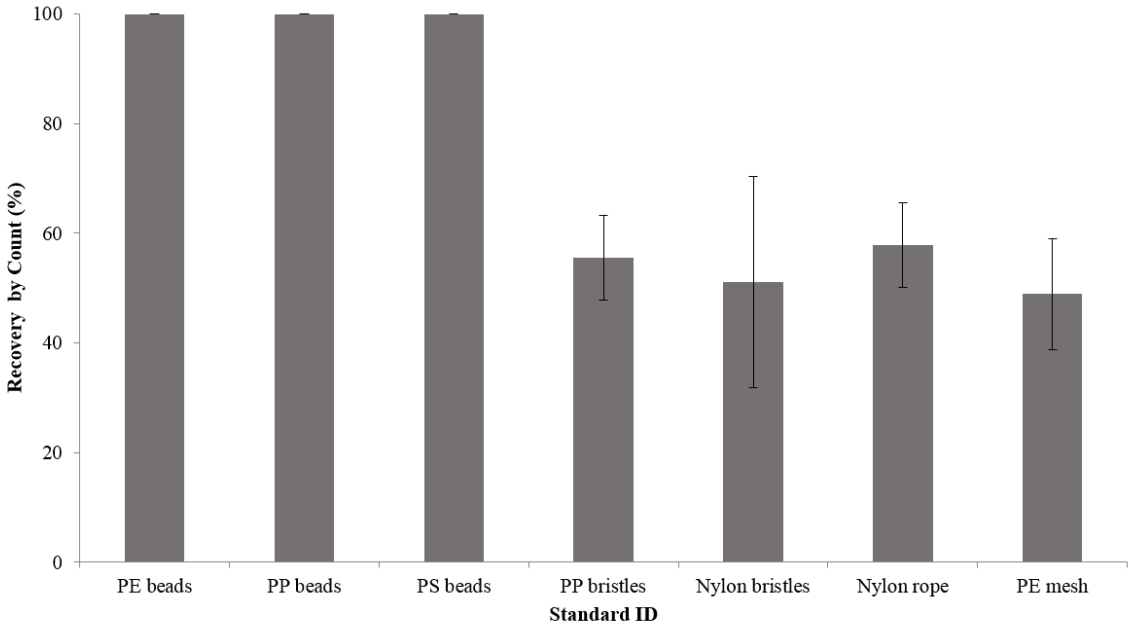


Figure 4-2 Recovery tests for density separation using various types of microplastic standards: purchased microbeads (polyethylene, PE; polypropylene, PP; and polystyrene, PS), and fibre-like fragments produced in the lab (PP bristles from a cleaning brush, nylon bristles from a toothbrush, nylon rope, and PE mesh packaging).

4.3.2 Microplastic categories

Suspected MPs were observed in all size fractions and were classified into three broad categories: (1) pellets, (2) fibres, and (3) fragments (**Figure 4-3**).

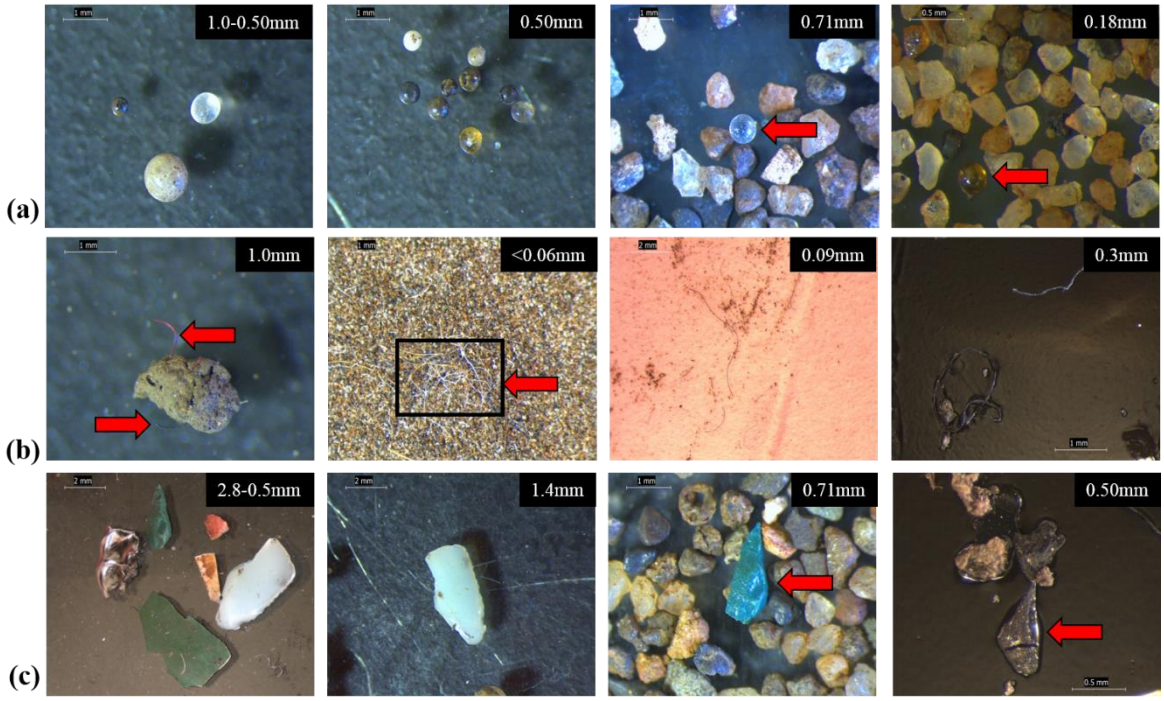


Figure 4-3 Light microscopy images of suspected microplastics in size-fractionated sediment samples from the River Kelvin in suspended and settled material before chemical characterisation. Items shown are: pellets (a), fibres (b) and fragments (c).

Micropellets

At stage 1, five micropellets were observed in suspended material in SE1 only (**Table 4-1**), but these were determined to be non-plastic at SE2. Visually, these pellets were dark-coloured and similar in appearance to those reported in a previous study in the St. Lawrence River (Castañeda et al. 2014). Pellets in the St. Lawrence River were determined to be polyethylene microbeads based on chemical characterisation by differential scanning calorimetry, thus suspended pellets in the River Kelvin were suspected to be also MPs. However, SEM-EDS analysis performed here showed suspended pellets were primarily metallic (**Figure 4-4**). The physical similarities but differing elemental compositions between the two studies indicate that non-MP pellets can be easily mistaken for MPs by visual inspection alone. The absence of primary MPs in this study contrasts with reports from earlier freshwater studies in urban catchments that found primary MPs to be more common than secondary forms based on visual and chemical characterisation (Zbyszewski and Corcoran 2011; Eriksen et al. 2013; Castañeda et al. 2014; Hurley et al. 2018; Peng et al. 2018). The high recovery rates for pellets from the validation tests provided confidence that, although no MP pellets were isolated from the environmental samples for this study, this was likely due to their absence from the site and not due to extraction error.

Table 4-1 Microplastic counts in River Kelvin sediment sampled December 17, 2015 (SE1) and February 15, 2016 (SE2) by category, and total counts and abundance aggregated across all size fractions for stages 1 (visual characterisation) and 2 (chemical characterisation).

Stage	Event	Sediment Weight, Dry (g)	Microplastics Count (n)					Abundance (items kg^{-1})
			Pellet	Fibre	Fragment	Other	Total	
Visual (Stage 1)	SE1	441.49	5	64	23	5	97	220
	SE1 Blanks (n=2)	0	0	3	0	0	3	
	SE2	254.48	0	106	8	0	114	448
	SE2 Blanks (n=4)	0	0	3	0	0	3	
Chemical (Stage 2)	SE1	441.49	0	64	7	0	71	161
	SE2	254.48	0	106	4	0	110	432

Visual examination revealed that micropellets were the predominant type of MPs in settled material by count across all size fractions for December and February samples, respectively (**Table D-1**). Settled micropellets consisted mostly of dark spheres similar to suspended ones, with a few clear and white- or cream-coloured pieces (**Figure 4-3a**). Micropellets were present mainly in the mid-range particle size fractions (0.25-0.7 mm). These were also present in clusters or aggregations of pellets that appeared to have been fused or melted together. Owing to their physical resemblance to micropellets observed in previous studies (Castañeda et al. 2014), an aliquot of settled pellets representing varying colours and sizes, was analysed by SEM-EDS to assess whether they were high-density MPs or non-plastic. The chemical composition was determined to be mostly metallic for dark pieces, while light-coloured pellets were mostly silica (**Figure 4-4**). While these micropellets were not MPs and therefore not the focus of this study, their high concentrations might warrant further evaluation to determine source of origin since they do not occur naturally in the aquatic environments. For example, aluminium silicate pellets could reflect coal fly ash as observed in the Laurentian Great Lakes (Eriksen et al., 2013), while other metallic pellets could be contaminants related to mining and industrial activities similar to those observed in other UK rivers (Rees et al., 1999). If similar in size, shape, and colour as their MP counterparts, these micropellets could also be harmful to the aquatic fauna if ingested. It is also important to be aware of their presence as they could be mistaken for MPs by visual inspection, especially if extracted by density separation as here. As metals have higher density, it would be expected that DS would not extract these materials. In this study, the five pellets in SE1 extracted by DS at stage 1 may be explained by the presence of a porous surface that was only evident during examination of structural composition in SEM-EDS images.

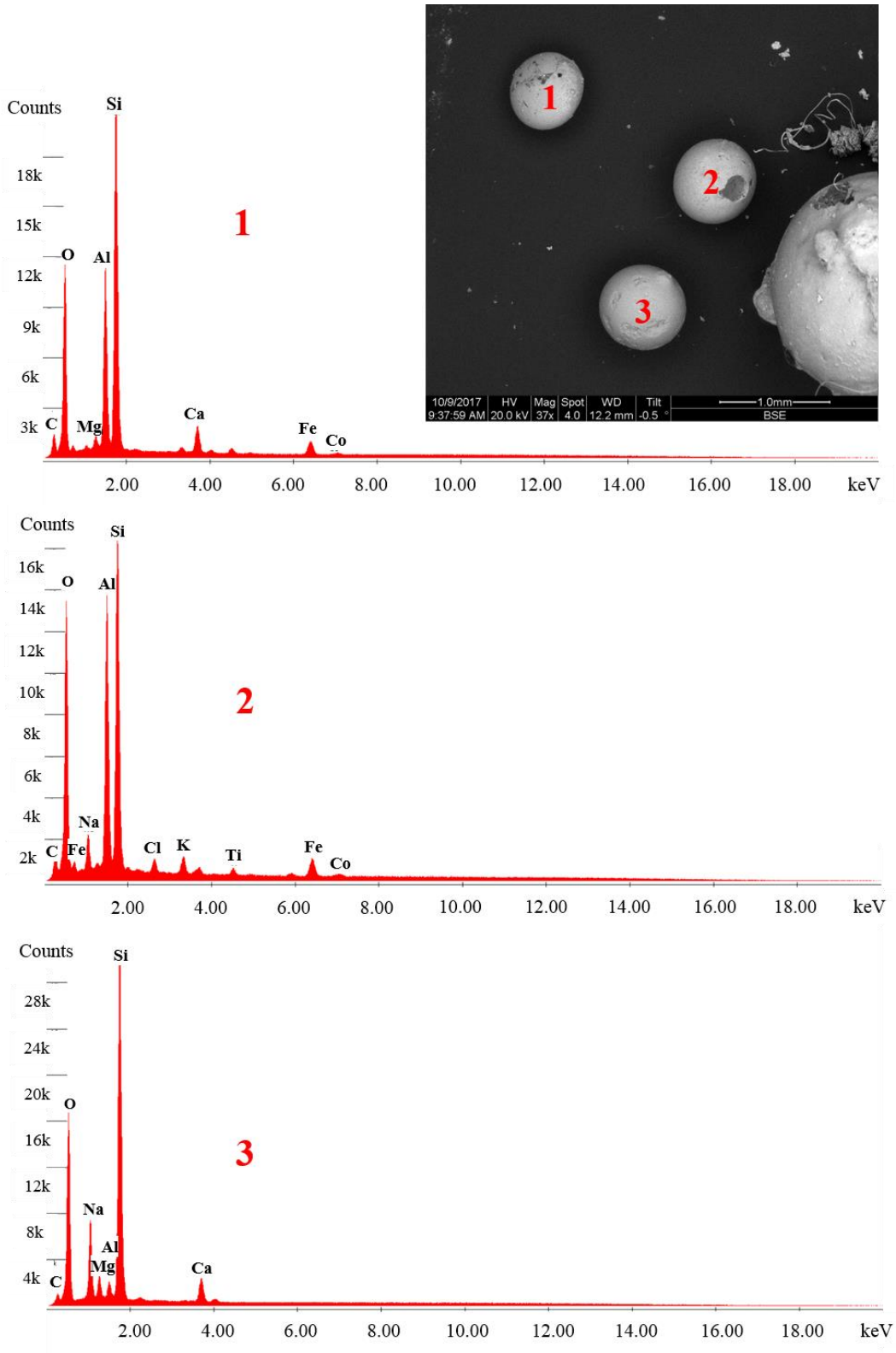


Figure 4-4 Backscattered electron image and elemental spectra for common micro-pellets observed in River Kelvin sediment. Pellets were determined to be non-plastic based on absence of a strong carbon signal.

Microfibres

Fibres were the most abundant type of suspended microdebris (**Table 4-1**), consisting primarily of coloured pieces (i.e. black or dark blue, light blue, and red). Micro-fibres of similar characteristics were observed in other freshwater ecosystems (Ballent et al. 2016), where fibres <2 mm identified visually with a stereo microscope were found to be the predominant type of MPs, alongside fragments in the same size range. In the River Kelvin sediment, fibres were observed in isolation, in clusters and embedded in sediment grains (**Figure 4-3b**). Microfibres were observed mostly in the lower size fractions (<0.090), with the <0.063 mm size fraction containing nearly 34% and 44% of total fibres in SE1 and SE2 samples respectively (**Table D-1**). However, their small sizes and tendency to cluster made it challenging to identify and enumerate visually by light microscopy, especially in the <0.06 mm fractions (**Figure 4-3b**), potentially leading to their underestimation. No fibres were observed in settled material after DS.

During SEM-EDS analysis at stage 2, fibres exhibited a strong C peak, sometimes accompanied by a smaller O peak (**Figure 4-5**). Therefore, fibres could not be dismissed as non-plastic from their density and chemical composition, resulting in equal counts at stages 1 and 2. Fibres comprised approximately 88% and 95% of all plastic pieces in SE1 and SE2, respectively, in the final enumeration. However, other non-plastic fibres such as cellulose-based ones can exhibit a similar structure and C signal (Remy et al. 2015), and SEM-EDS does not allow for distinction between them (**Figure 4-5**). Spectroscopy analysis via FTIR and Raman has been used successfully for further isolation of MP from non-MP fibres (Remy et al. 2015), highlighting the need for advanced chemical characterisation tools for proper MP quantification, especially in the case of fibres.

Similarly, others have reported the predominance of fibres (Ballent et al. 2016; Su et al. 2016), especially in systems associated with wastewater treatment as such fibres typically break off synthetic textiles and are released via household sewage (Browne et al. 2011; Magnusson and Nóren 2014). While the selected site in the River Kelvin is not located near a discharge pipe from a wastewater treatment facility, it has been suggested that fibres can be transported for greater distances (Ballent et al. 2016), thus their presence may be attributed to distant inputs upstream from the study site. Conversely, a portion of fibres observed in the samples may be explained by atmospheric fallout of airborne fibres, which can be corroborated by fibre content in rooftop samples collected in urban Paris (Dris et al. 2015) and the presence of microfibres in our procedural blanks. While fibre content in blanks could be a result of aerial deposition of fibres released during wear and tear of lab gear, additional deposition of airborne materials into the open channel may occur in the field and account for a portion of fibres observed in river sediment. Furthermore, fibre content in drinking tap water tested in multiple countries (Kosuth et al. 2018) may suggest potential background contamination of fibres even in water purification systems, but this was not tested here and limited studies on MPs in drinking water are currently available.

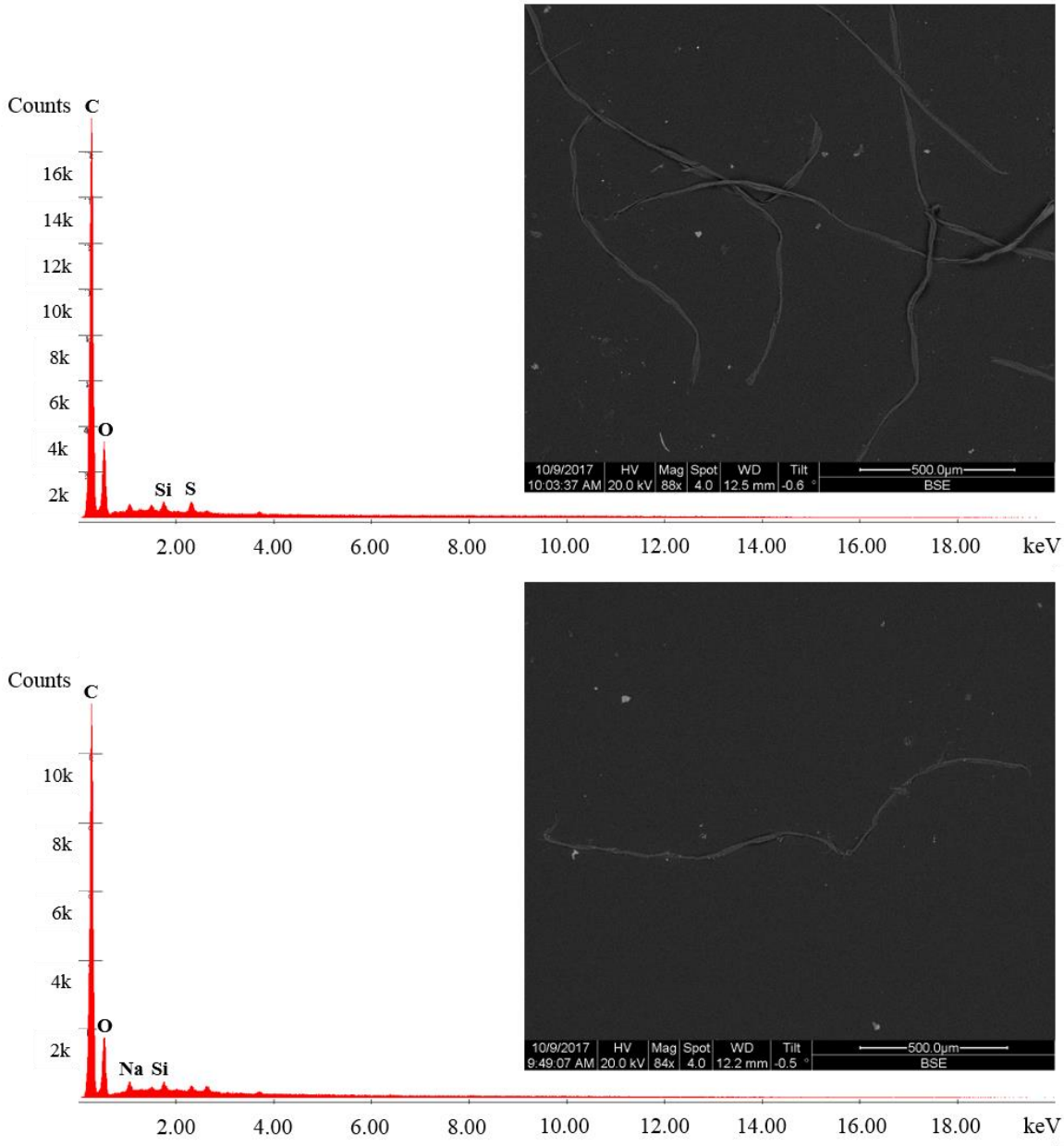


Figure 4-5 Backscattered electron image and elemental spectra for common micro-fibres (top) observed in River Kelvin sediment and a 100% cotton fibre standard (bottom). Fibres exhibited a strong carbon signal, but MP could not be discriminated against cellulose fibres.

Microfragments

The third category comprises fragmented or flake-like pieces that had uneven edges and appeared to have broken off larger pieces. Suspected MP fragments were observed in suspended and settled material and consisted mainly of coloured pieces (**Figure 4-3c**). Counts varied between sampling events and quantification stage and although the highest counts were observed in the 0.71 mm size fraction at stage 1, this was not the case for the final counts, and they did not seem to concentrate around a specific size fraction in a discernible pattern. Because high-density polymers can be present in the environment, all settled fragments that physically resembled plastic materials were counted as suspected MP at stage 1 and analysed for chemical composition. Unlike pellets that consistently had

little to no C, and fibres that consistently were mostly C, SEM-EDS signals for fragments were more varied and complex.

Suspended flake-like fragments with a strong C signal (**Figure 4-6a**) became visible only during SEM-EDS imaging. This is likely explained because these pieces were captured on the filter paper after DS, and, while not visible under light microscopy, they were transferred onto the adhesive while attempting to transfer other materials like fibres using the "pressing" method. Furthermore, electron microscopy enables greater resolution than light microscopy, making SEM-EDS a powerful tool for detection of smaller pieces like these that may be overlooked by visual inspection, and highlights the detection limits of visual techniques.

Other suspended fragments showed a strong C peak, but exhibited additional elemental signals including Ti, Br, and Si (**Figure 4-6b**). These pieces were counted as MPs, due to their strong C signal and low densities, but further analysis via spectroscopy tools (e.g. Raman, FTIR) should be employed in these cases to identify the type and source of these (and similar pieces) to be conclusive. Only one of ten settled MP fragments showed a strong C signal in the SEM-EDS analysis (**Figure 4-6c**). This may indicate high-density plastic fragments, for example, polyvinyl chloride from construction applications, or polytetrafluoroethylene and engineering polyesters from industrial applications that would need heavier liquids to be extracted (Hidalgo-Ruz et al. 2012). The remaining settled pieces, while initially expected to be plastic due to their bright colours and shapes, showed no carbon signals at stage 2 (**Figure 4-6d**) and therefore were rejected from final counts.

Fragments comprised 12% and 5% of total MP counts in SE1 and SE2, respectively (**Table 4-1**). While most studies report either pellets or fibres as the predominant forms of MP debris, and a diversity of fragments generally have been observed across rivers and lakes worldwide, a few studies have reported fragments as the predominant form of these materials in freshwaters systems (Vianello et al. 2013; Wagner et al. 2014; Hurley et al. 2018; Wen et al. 2018; Shruti et al. 2019). Their presence in the catchment may be a result of historical industrial activities or from the fragmentation of plastic litter as the River Kelvin catchment is an area for multiple recreational activities and the sampling site is located underneath a heavily transited bridge near tourist attractions. However, as fragments can originate from the breakdown of larger pieces, their sources may be harder to trace as they are likely to result from non-point pollution, such as rainwater runoff to road drainage systems, losses from landfill sites, riverbanks and floodplains (Kataoka et al. 2018). This is particularly important in MPs research as fragments may become more abundant if plastic litter already present in the environment continues to degrade into smaller fractions, and as MPs can further fragment into nanoplastics. Thus, more information on degradation or fragmentation rates of different polymers may play a key role in understanding this category (Hidalgo-Ruz et al. 2012).

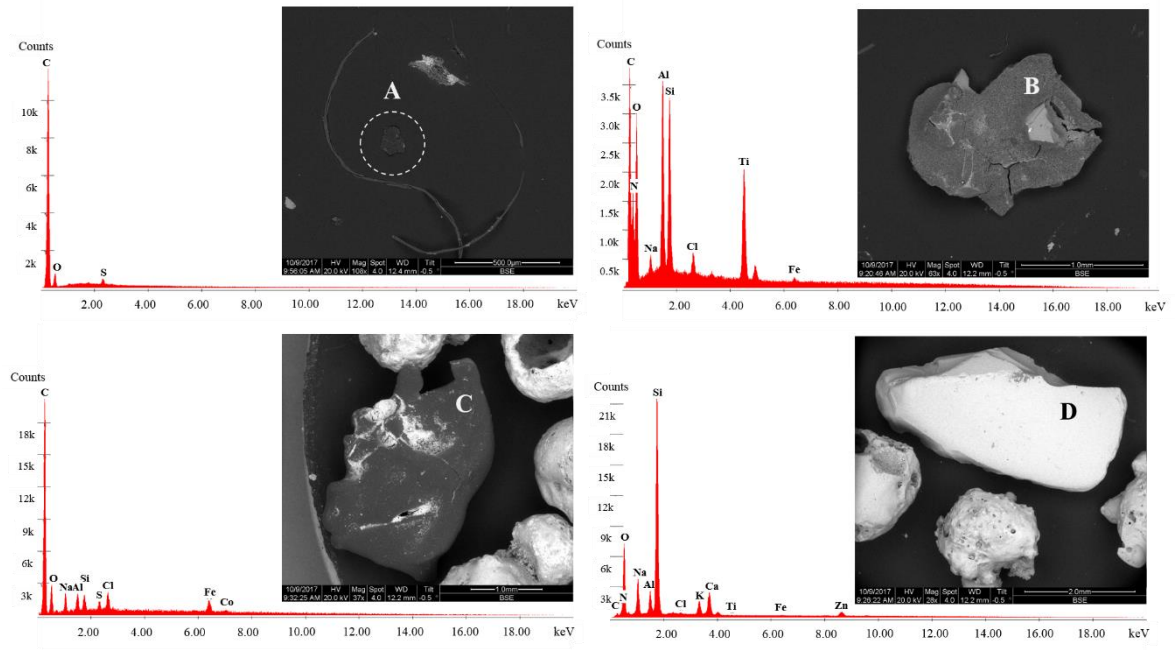


Figure 4-6 Backscattered electron image and elemental spectra for common micro-fragments observed in River Kelvin sediment showing floated microplastics (a) and (b), settled microplastic (c), and settled non-microplastic (d) pieces. Pieces were identified as microplastic on the basis of a strong carbon signal.

4.3.3 Microplastic abundances

Suspected MPs abundance at identification stage 1 supported initial estimates of 220 items kg^{-1} of dry sediment in SE1 and 448 items kg^{-1} of dry sediment in SE2. Final MP abundance at stage 2 were 161 and 432 items per kg of dry sediment in SE1 and SE2 samples respectively (**Table 4-1**). These concentrations are within ranges observed in other European sites. For example, sediment samples collected from German rivers and inspected visually (Wagner et al. 2014) and chemically (Klein et al. 2015) found 34-64 items kg^{-1} dry weight in the Rivers Elbe, Mosel, Neckar, and Rhine, and fragments accounted for 60% of total microplastics, with the remainder being fibres (Wagner et al. 2014). However, abundances can be spatially and temporally variable, with other sediment samples from the Rhine yielding 228-3,763 items kg^{-1}, and further 786-1,368 items kg^{-1} in the River Main (Klein et al. 2015). At these sites, the relative abundance of spheres and fragments compared to other shapes was highest in the 63−200 µm and 200-5000 µm size fractions, respectively, while fibres were most abundant in size fractions <200 µm compared to their concentration in higher size fractions (Klein at el. 2015). In addition, sediment MP abundances in the River Thames were found to range from 18.5±4.2 to 66±7.7 particles 100 g^{-1} (equivalent to 185 and 660 particles kg^{-1}) of sediment across four sites, with fibres as the main type in three sites and fragments in the fourth, based on visual and chemical characterisation (Horton et al. 2017). High MP contamination was observed in multiple river channels in the Mersey and Irwell catchments in Northwest England, where 517,000 particles m^{-2} were observed on the River Tame (Hurley et al. 2018).

Concentrations in river sediments in non-European regions are generally higher compared to those observed in this study and are usually associated with urban and densely-populated areas. For

example, averages of 802±59.4 MPs kg^{-1} were observed across seven urban rivers in Shanghai (Peng et al. 2018), with greater concentrations in densely populated areas compared to rural areas. In Changsha, concentrations ranged from 307.55±94.73 to 580.79±310.35 MPs kg^{-1} in urban waters across four tributaries to the Xiangjiang River that serves 7 million people with drinking water, although the relationship between MP abundances and distance to urban centers was not significant (Wen et al. 2018). Concentrations ranging from 833.33±80.79 to 1,633.34±202.56 kg^{-1} were observed in an urban river system in Central Mexico, with films and fragments comprising the bulk of pieces (Shruti et al. 2019).

The relative abundance of secondary MP types observed here is also consistent with those from other freshwater studies conducted in Lake Hovsgol (Free et al. 2014), the Raritan River (Estahbanati and Fahrenfeld 2016), and urban Paris (Dris et al. 2015), although this comparison can only be expressed qualitatively as different measurements and units were used. Methods and measurement units used in reporting results need harmonising for improved risk assessment and to facilitate discussion across studies. Nevertheless, the predominance of secondary MPs in the River Kelvin and other freshwater catchments supports the general assumption that most MPs in the environment originate from the breakdown of larger pieces (Duis and Coors 2016). Coloured pieces were more frequent than white and translucent pieces (**Figure 4-7**), but further data is needed to determine whether this is an accurate reflection of their greater abundance in the environment, or if this is attributed to selection bias. Indeed, it has been suggested that fibre-like and bright-coloured pieces may be easier to find (Hidalgo Ruz et al. 2012; Cole et al. 2014) and could be a source of analytical bias.

As the sampling site is a low-energy zone where sediment deposition tends to occur, the abundance of MPs here may support previous interpretations that processes affecting deposition of fine sediment similarly influence MPs (Vianello et al. 2013; Nizzetto et al. 2016), and may explain why fibres were more abundant and concentrated in the lower size fractions. Nevertheless, the distinctly different abundances observed between December and February samples in the River Kelvin suggests that high local variability can be expected, likely because MP contaminants encompass a wide array of highly-diverse particles and thus will not be evenly distributed in space and time. The use of only one sampling site is a potential limitation of this study given the expected spatio-temporal variability of MPs in nature and further spatial sampling and comparative data from the site and the local catchment are needed to improve our understanding of MP behaviour and distribution in this and similar freshwater systems. In addition, it is crucial to increase the spatial coverage of freshwater surveys through research like this, and the comparability across studies to fully understand this variability (Turra et al. 2014) and improve reliable assessment of their distribution and abundance in aquatic environments.

This research shows that freshwater river sediments close to marine estuary systems contain MPs, with fibres numerically dominant, and thus it is likely that freshwater systems are a feeder of marine MPs, mobilised for example to the marine environment by large flows (Nizzetto et al. 2016; Hurley et al. 2018). Moreover, the fate of MPs in these systems may be influenced by the association of different MP types and sizes with different sediment grain size fractions and some MPs may be retained (Nizzetto et al. 2016). Thus, consideration of different particle-size fractions and areas

where sediment accumulates is needed in river MP studies to improve understanding of MP emissions to oceans.

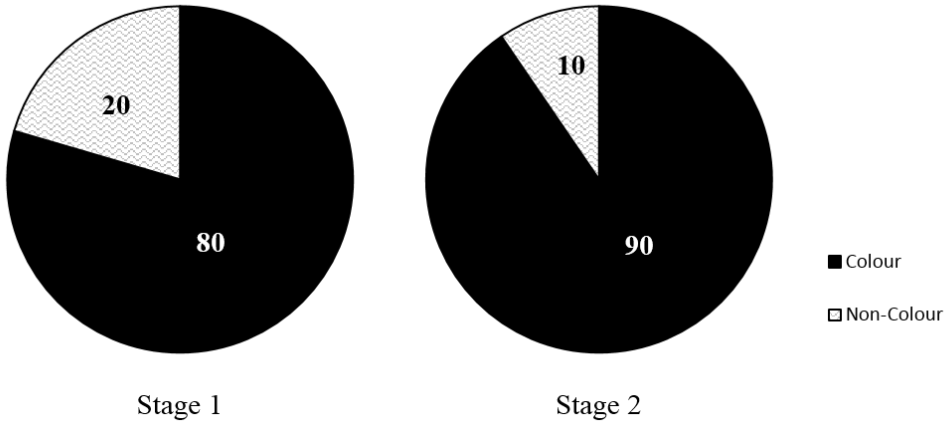


Figure 4-7 Percentages of coloured and non-coloured (i.e. white and translucent) pieces observed in River Kelvin sediment samples at each characterisation stage (data is pooled for both sampling events).

4.3.4 Visual vs chemical characterisation

Counts and relative abundance of suspected MP types were used to compare the efficacy of visual and chemical characterisation techniques to discriminate plastics from other non-plastic microdebris and the sediment matrix before and after SEM-EDS analysis. Visually, identification of pieces that were different than sediment grains was possible by light microscopy although this was increasingly difficult in the fractions smaller than 0.125 mm due to decreasing resolution, and it was nearly impossible to distinguish plastic from non-plastic microdebris. As a result, visual characterisation may lead to overestimation of MP pieces due to misidentification, because floatation of non-polymer microdebris can occur and because non-plastic pellets and fragments can be easily confused for MP given their physical similarities. Visual inspection is often used in methodological approaches for initial enumeration and identification (Hidalgo-Ruz et al. 2012; Blair et al. 2017). However, heavy reliance on the visual and manual components at nearly every step of the process can introduce potential for selection bias (Cole et al. 2014) and is limited by what is reasonably visible with or without the aid of a microscope. While this detection limit will depend on the individual doing the identification, it is recommended that visual characterisation is not used for pieces smaller than 0.5 mm (Hidalgo-Ruz et al. 2012), a limit much higher than the lower limit set by sampling (e.g. 0.3 mm for neuston nets) and filtration (e.g. 0.7 micron for glass fibre filters) methods, including those used in this study.

Here, the chemical composition data from SEM-EDS was useful mainly for separation of non-plastic pellets and fragments in both suspended and settled material, but it was not useful for MP fibre identification. Further analysis by spectroscopy techniques such as Raman and FTIR-ATR (Blair et al. 2017) are likely necessary for proper MP fibre enumeration. While chemical characterisation by

SEM-EDS and other complementary techniques like Raman and FTIR spectroscopy can aid to overcome detection limits and misidentification from visual characterisation (Wesch et al. 2016), it is important to note their limitations. First, these techniques can be extremely time-consuming and may be costly. For similar logistical reasons, it was possible only to analyse a microfibre sub-aliquot via SEM-EDS in this study. Care was taken to ensure that the sub-aliquot was representative of all types, colours, and size categories, but extrapolation of SEM-EDS results to the rest of the sample is undertaken visually and could result in some MP items being overlooked or misidentified. Second, chemical characterisation may be also subject to selection bias as MP specimens needed to be isolated from other media and manually transferred to the instrument for analysis, depending on the ability of the researcher to first find these pieces visually. Lastly, instrument aided detection is also subject to size limitations. For Raman and FTIR, this is considered to be in the range of 0.5 and 10 μm, respectively (Hale 2017), although this may vary according to the equipment employed.

A combined approach that uses visual and multiple chemical characterisation techniques can address some of these methodological limitations. Combined or stepwise approaches are becoming more common in recent routine testing as a way to optimise extraction and characterisation methods and reduce analytical errors (Hidalgo-Ruz et al. 2012; Horton et al. 2017). Further, new studies are recognising the impact of visual reliance on size limitations and proper MP identification and are using advanced FTIR mapping techniques to develop automated methods (Primpke et al. 2017). This is an important step forward in method development because a lower size limit for MPs is yet to be established. In addition, automated methods will be crucial for emerging nanoplastic (<100 nm) research that may become more abundant in the environment as their use increases in future trends in technological applications and as macro- and microplastic waste continues to degrade (Koelmans et al. 2015).

4.4 Conclusions

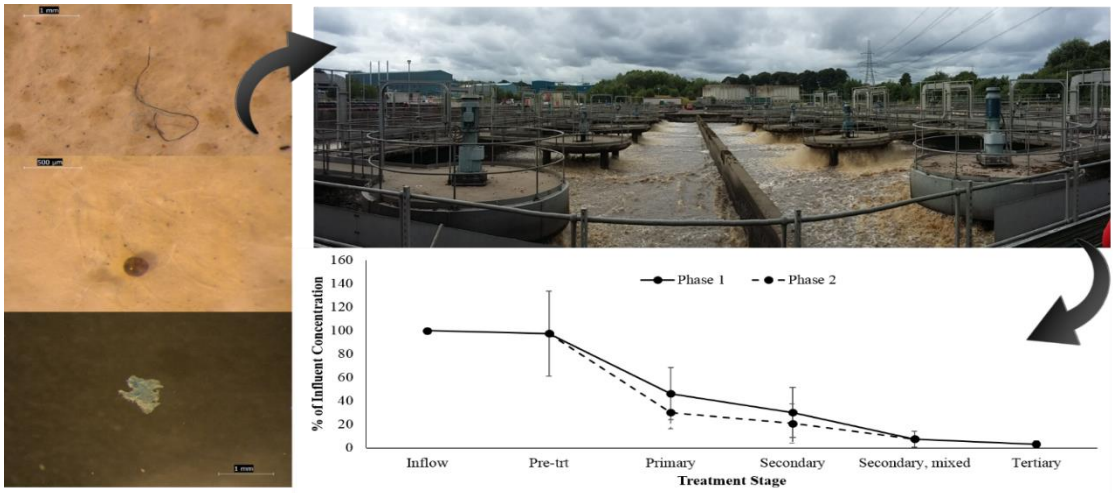
While MP pollution research is experiencing rapid development, research remains largely skewed towards marine systems with limited information for freshwater river compartments. As rivers receive anthropogenic waste inputs from the land they drain, they can act as important conduits of MPs from land-based sources to oceans and thus cannot be separated from marine MPs research. Therefore, this study contributes to a currently limited body of work exploring the concentration and composition of MPs in freshwater river sediment in close proximity to the marine environment. Furthermore, previous studies usually explore the correlation between MP concentrations and basin characteristics to identify potential sources, but this is one of the first to explore the associations of different types of MPs with different grain size fractions. This information contributes to understanding of the behaviour and fate of MPs in these systems to identify potential control points.

Results corroborate the ubiquity of MPs and suggest the predominance of secondary MPs, but high variability was observed in MPs concentrations across sampling events during the same season. Fibres were always the dominant type of plastic and while often associated with sewage discharge, their presence in this site suggests a greater contribution of other pathways, such as atmospheric deposition or in-stream transport. Nevertheless, this study focussed only on the exposed sediment fraction and a single sampling point, which are potential limitations; therefore, future work should

expand on spatial sampling and incorporation of other environmental compartments to assess the extent of their spatio-temporal variability and the potential for storage vs transport of MPs in these systems. However, often it may not be possible for researchers to include the samples needed for a comprehensive assessment of all liquid, solid, and gaseous fractions, thus research efforts should also aim to unify methodology for improved inter-comparison of available freshwater studies. Currently, methods can be subject to both under- and overestimation of different types of MPs, limiting comparability and potentially leading to inaccurate assessment of MPs pollution, hindering risk assessment and possibly resulting in mitigation efforts that are largely misdirected. Further work is currently underway to examine the spatio-temporal distribution and chemical composition of MPs in a larger freshwater river system in the same catchment location reported in this paper. This study contributes to this further spatio-temporal survey by establishing a particle-size fraction profile of possible MPs in the catchment and refining the techniques needed to improve their extraction and identification.

The information collected from the River Kelvin evidenced the ubiquity of MPs in the environment. However, the chemical characterisation used in this part of the study was not sufficient to infer sources of MPs in the catchment, which may be due to a combination of the historical legacy of industrial and sewage discharges to the river, and recent inputs from CSO discharges, urban runoff, and recreational activities. Fibres were the most abundant type of plastic, which tend to be mainly associated with washing machine and wastewater effluent. Therefore, the need to examine the role WWTPs as pathways of fibres and other MPs was recognised. These facilities may act as transport vectors but also as the first filters for MPs passing through anthropogenic water systems.

5 Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period



Statement of authorship

RMB was responsible for the conception of the experimental design with feedback from supervisors, liaising with Scottish Water for access to site and flow data, and performed all the experimental work and analysis and interpretation of data. RMB drafted the article for submission. SW and CGL contributed to the planning of the experiment and editing of the manuscript. The enclosed paper has undergone revisions with comments from three anonymous reviewers and the final version was accepted for publication July 22 2019. An addendum written by RMB is included at the end of the chapter for additional data on colour repartition for Daldowie samples and MP quantification for two activated sludge samples obtained from Shieldhall, a nearby secondary WWTP in the same river catchment.

Chapter introduction

The following publication summarises the work conducted at the selected WWTP and was planned following the results from River Kelvin sediments that indicate that there are numerous MPs in these systems. During the first study, the mode of entry of these particles to the catchment could only be speculated. Therefore, I wanted to start by exploring the role of point-source pollution to rivers. The main focus of this part of the project was to assess the spatio-temporal distribution of MPs in a tertiary treatment system and the efficiency of the system to remove these materials by comparison of daily incoming and outgoing MP loads.

Citation

Blair RM, Waldron S, Phoenix VR, Gauchotte-Lindsay C (2019) Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. Water Res DOI: https://doi.org/10.1016/j.watres.2019.114909
Pre-print (DOI: 10.31223/osf.io/ez5gm) available 8/3/19: https://eartharxiv.org/ez5gm/

Abstract

Microplastics (MPs, <5 mm in size) are classified as emerging contaminants but treatment processes are not designed to remove these small particles. Wastewater treatment systems have been proposed as pathways for MPs pollution to receiving waters but quantitative and qualitative data on MP occurrence and transport remains limited, hindering risk assessment and regulation. Here, for the first time, the stepwise abundance and loading of MPs (60-2800 µm) in a tertiary wastewater treatment plant in the UK was assessed by sampling from May 2017 to February 2018. Microplastics were found in all sampling campaigns, with an average inflow of 8.1×10^8 (95% CI, 3.8×10^8 to 1.2×10^9) items day^{-1}. Their prevalence decreased from influent to final effluent. Overall abundances decreased on average by 6%, 68%, 92%, and 96% after the pre-treatment, primary, secondary, and tertiary treatment stages respectively, although considerable variability occurred throughout the year. Sufficient particles remained in the treated effluent to generate an average discharge of 2.2×10^7 (95% CI, 1.2×10^7 to 3.2×10^7]) particles day^{-1} to the recipient river. Secondary MPs were predominant, while primary MP abundances were minimal. Fibres comprised 67% of all items, followed by films (18%) and fragments (15%). Chemical characterisation confirmed the presence of different types of polymers, with polypropylene fibres and fragments most abundant (23%). This research informs understanding of how wastewater effluent may channel MPs to the natural environment and their composition, and helps understand control points for optimising advanced treatment processes.

5.1 Introduction

Microplastics (MPs; <5 mm) are ubiquitous in the environment and may pose a threat to biota and humans (Anbumani & Kakkar 2018), thus are classed as emerging contaminants but remain unregulated by water quality standards. This may be largely because they have not been fully assessed due to their heterogeneous nature and high spatio-temporal variations, even within localized environmental compartments. Furthermore, a lack of standardized protocols leads to limited comparability across available surveys and a lack of guidelines to monitor MPs in aquatic systems. Current empirical data is still too limited to fully understand the extent of their pollution and the severity of their threat, making it difficult for regulators to determine what types of MPs need to be prioritised in monitoring programmes and where controls should be implemented. Nevertheless, similar to other anthropogenic contaminants, 80% of MPs are considered to originate from land-based sources (Rochman et al. 2015). Therefore the role of wastewater treatment plants (WWTPs) as potential barriers of MP pollution should be considered, as they are important links between the anthropogenic and natural environments (Ou & Zeng 2018).

Wastewater treatment systems are designed to remove contaminants from household and trade effluent, so their role in MPs removal has been generating increasing attention, yet they remain largely unexplored (**Table 5-1**). The majority of available studies quantify MPs in secondary effluent, with fewer studies considering tertiary treatment plants (**Table 5-1**). Here, secondary treatment refers to biological wastewater treatment (e.g. activated sludge) resulting in the separation of decanted effluent and sludge containing microbial biomass (European Environment Agency 2019). Tertiary or advanced treatment refers to post-secondary polishing steps (e.g. chemical removal,

advanced filtration) to eliminate pollutants not removed by secondary treatment (European Environment Agency 2019). Current understanding suggests that a mixture of primary and secondary MPs may be entering the treatment facilities daily, at varying levels of pollution (Sun et al. 2019). Microplastic concentrations in raw wastewater are reported so far to range from <1 particle L^{-1} as observed by multiple studies (**Table 5-1**), to 18,285 particles L^{-1} reported in a secondary treatment site in Denmark (Simon et al. 2018). Conversely, effluent concentrations between 8 x 10^{-4} (Magnusson and Noren. 2014) and 447 (Simon et al. 2018) particles L^{-1} have been observed in secondary WWTPs, and between 0 (Carr et al. 2016) and 51 particles L^{-1} (membrane bioreactor, MBR; Leslie et al. 2017) after advanced treatment (Sun et al. 2019), with larger facilities likely discharging higher loads (Mason et al. 2018). While the WWTP literature has grown over the past two years, each study differs in methodologies (e.g. sampling volumes, detection limits), plant capacity, and type of treatment technologies and stages examined. Therefore, it is difficult to determine what variation across studies is due to site differences or analytical bias, limiting comparability of findings and comprehensive understanding of the occurrence and fate of MPs in these systems.

Comparison of influent vs effluent concentrations is a common approach to estimate removal efficiencies, which range between 40% and 99.9% (**Table 5-1**). While absolute values may be difficult to compare, reporting of removal percentages may improve intra-study comparisons, but not all studies report this. Despite high retention efficiencies, low concentrations in final or treated effluent may represent daily releases of millions of MPs when scaled for the discharge volumes (Mason et al. 2016; Murphy et al. 2016). For instance, concentrations of 2.5 x 10^{-1} and 4 x 10^{-3} particles L^{-1} in final effluent, equated to discharges of 6.5 x 10^{7} and 5 x 10^{4} MPs day^{-1}, respectively in secondary treatment plants in Scotland, UK (Murphy et al. 2016) and San Francisco, USA (Mason et al. 2016). Microplastic discharges from WWTPs appear highly variable, and treatment procedure employed at the facility is presumed to be crucial in their retention.

Table 5-1 Summarised research from 2011 and 2019 on MPs in WWTPs

#	Location	Treatment Type	Plant size (p.e.)	Sites	Sample Volume (L)	Stages Sampled	Biosolid Samples	Analytical Method	Size Range (µm)	Effluent Concentration (count L^{-1})	Removal (%)
1	Australia	Tertiary		2	0.75	Effluent	None	FTIR	<1000	1	
2	Sweden	Secondary	$1.4x10^4$	1	2 - 1000	Influent, final effluent	Sewage sludge	Visual sorting; FTIR	>300	8×10^{-3}	99.9
3	France	Secondary		1	0.05	Influent, primary, final effluent		Visual sorting	100-5000	14 - 50	83-95
4	USA	Secondary & Tertiary		7	189000 - 232000	Influent, primary, secondary, final effluent	Sewage sludge, activated sludge	Visual sorting; FTIR	45-400	8×10^{-4}	~99.9
5	USA	Secondary & Tertiary	3.5×10^3 - 5.6×10^7	17	500 - 21000	Final effluent	None	Visual sorting	>125	5×10^{-2}	
6	USA	Secondary & Tertiary		3	1 - 38	Influent, pre-treatment, primary, secondary, final effluent	None	Visual sorting	20-4750	1.4 - 2.6	95.6-99.4
7	Scotland	Secondary	6.5×10^5	1	30 - 50	Influent after screens, pre-treatment, primary, final effluent	Grit and grease, sludge cake from centrifuge	Visual sorting; FTIR	> 65	2.5×10^{-1}	98.41
8	USA	Secondary & Tertiary		8	2-hr composite	Final effluent	None	Visual sorting	125-355	4.7×10^{-2} - 1.9 $\times^{-1}$	
9	USA	Secondary	6.8×10^5	1	2-24 hr composite	Final effluent	None	Visual sorting; Raman; FTIR	125-5000	0.3 - 2.4	

Table 5-1 continued Summarised research from 2011 and 2019 on MPs in WWTPs

#	Location	Treatment Type	Plant size (p.e.)	Sites	Sample Volume (L)	Stages Sampled	Biosolid Samples	Analytical Method	Size Range (µm)	Effluent Concentration (count L^{-1})	Removal (%)
10	Netherlands	Secondary & Tertiary		7	2	Influent, final effluent	Sewage sludge	Visual sorting; FTIR	10-5000	9 -91	
11	Germany	Secondary & Tertiary	7.0×10^3 - 2.1×10^5	12	390 - 1000	Final effluent	Sewage sludge	Visual sorting; FTIR	20-5000	1×10^{-3} - 9	~97
12	Finland	Tertiary	5×10^4 - 8×10^5	4	0.4 - 1000	Influent, final effluent	None	Visual sorting; FTIR	20->300	5×10^{-3} - 3×10^{-1}	40-99.9
13	Finland	Tertiary	8×10^5	1	0.1 - 1000	Influent, pre-treatment, secondary, final effluent,	Excess sludge, dry sludge	Visual sorting; FTIR	20->300	7×10^{-1} - 3.5	>99
14	Australia	Primary, Secondary & Tertiary	1.5×10^5 - 1.2×10^6	3	3 - 200	Final effluent	None	Staining and visual sorting; FTIR	25-500	2.8×10^{-1} - 1.54	90
15	Canada	Secondary	1.3×10^6	1	1 - 30	Influent, primary, final effluent	Sewage sludge, activated sludge	Visual sorting; FTIR	1-65	5×10^{-1}	99
16	Finland	Secondary		1	4 - 30	Influent after screens, primary, final effluent	Activated sludge, digested sludge, membrane bioreactor sludge	Visual sorting; FTIR, Raman	0.25-5000	4×10^{-1} - 1	98.3

Table 5-1 continued Summarised research from 2011 and 2019 on MPs in WWTPs

#	Location	Treatment Type	Plant size (p.e.)	Sites	Sample Volume (L)	Stages Sampled	Biosolid Samples	Analytical Method	Size Range (µm)	Effluent Concentration (count L^{-1})	Removal (%)
17	Denmark	Secondary & Tertiary		10	1 - 81.5	Influent after screens, final effluent	None	FTIR-FPA	10-500	54	99.3
18	USA	Secondary	180,000-53000	3	3.6-30	Influent; final effluent	None	Visual sorting; FTIR		1-30	74.8-98.1
19	Italy	Tertiary	1.2×10^6	1	30	Influent, after settler, outlet	None	Visual sorting; FTIR	63-5000	4×10^{-1}	84

*__1__, Browne et al. 2011; __2__, Magnusson and Noren 2014; __3__, Dris et al. 2015; __4__, Carr et al. 2016; __5__, Mason et al. 2016; __6__, Michielssen et al. 2016; __7__, Murphy et al. 2016; __8__, Sutton et al. 2016; __9__, Dyachenko et al. 2017; __10__, Leslie et al. 2017; __11__, Mintenig et al. 2017; __12__, Talvitie et al. 2017a; __13__, Talvitie et al. 2017b; __14__, Ziajahromi et al. 2017; __15__, Gies et al. 2018; __16__, Lares et al. 2018; __17__, Simon et al. 2018; __18__, Conley et al. 2019; __19__, Magni et al. 2019

The role of different treatment processes in removing contaminants from these systems can be assessed by a stage-wise inspection of MPs abundances during their passage through a single facility. Owing to challenges of sample collection and processing times, only a few studies have done this (**Table 5-1**), and stages sampled vary across studies. It appears that between ~63 and 98% of the removal can occur by the primary stage (Sun et al. 2019). Secondary treatment may reduce an additional 7 to 20% of MPs not captured by preliminary and primary treatment (Talvitie et al. 2017b; Ziahjaromi et al. 2017; Gies et al. 2018). The observation of MPs in different types of biosolids suggest that their removal during earlier stages is through their capture in various sludge fractions including grit and grease skimmings (Murphy et al. 2016), sewage sludge (Bayo et al. 2016; Murphy et al. 2016; Leslie et al. 2017; Mintenig et al. 2017; Li et al. 2018), and returned activated or excess sludge (Carr et al., 2016; Talvitie et al. 2017a; Lares et al. 2018).

While the nature of primary and secondary treatment is mostly consistent across studies, there is an array of advanced treatment techniques. Studies comparing MPs in tertiary vs. secondary effluent found that different advanced treatment technologies can further decrease MPs before discharge (Michielssen et al. 2016; Mintenig et al. 2017; Talvitie et al. 2017a,b; Ziahjaromi et al. 2017; Lares et al. 2018; Magni et al. 2019). Overall, MBR (Lares et al. 2018; Talvitie et al. 2017a) and advanced filtration technologies (Michielssen et al. 2016; Mintenig et al. 2017; Talvitie et al. 2017 a,b; Ziahjaromi et al. 2017; Magni et al. 2019) have been reported as effective means in reducing MPs from final effluent. Dissolved air flotation in Finland (Talvitie et al. 2017a) and reverse osmosis and decarbonation in Australia (Ziahjaromi et al. 2017) also showed high performance. However, in other studies, advanced treatment by gravity sand filtration (Carr et al. 2016) and MBR (Leslie et al. 2017) did not promote further reduction in particle concentrations. These different findings in advanced WWTP studies support the need for further research on a range of treatment technologies to produce a representative assessment of their role in removing MPs from wastewater. This information could help identify control points within these systems, and what development or modification of operational procedures may decrease MPs discharge to the recipient waters.

Further research of WWTPs is crucial in MPs research because wastewater is a complex and heterogeneous matrix, and pollution levels and removal efficiencies appear to exhibit high inter- and intra-site variability (Mason et al. 2017). Especially, empirical data are needed for multiple stages other than final effluent and to explore factors driving spatio-temporal variabilities. Here, a study was conducted in a WWTP in the UK (Scotland) to: (1) understand the inflow and outflow loading of MPs (quantity and composition) in a tertiary treatment plant, accommodating temporal variability, and (2) assess the stepwise effect of treatment stage on the distribution and fate of MPs sized between 60-2800 µm. To our knowledge, this is the first study to evaluate MPs in advanced treatment systems in the UK by long-term (i.e. 10 months) spatial sampling in a single facility.

5.2 Materials and methods

5.2.1 Study site and sampling

The study site was a tertiary wastewater treatment plant in Scotland, UK, with 184,500 population equivalents (p.e.) and receiving a mix of trade and domestic sewage. The plant consists of

preliminary treatment of wastewater by coarse screening (12 mm) and grit removal, primary settling tanks (phases 1 and 2), activated sludge treatment and clarification in final settling tanks (phases 1 and 2), and nitrification on plastic media trickling filters (**Figure 5-1**), with final discharge of treated effluent into a freshwater river. Phases 1 and 2 were created due to an expansion of the treatment plant. This splits the stream into parallel channels for primary and secondary stages but there is no difference in treatment between the two.

Sampling was conducted five times between May 2017 and February 2018: 19 May 2017 (sampling event, SE1), 13 July 2017 (SE2), 20 October 2017 (SE3), 11 January 2018 (SE4), and 16 February 2018 (SE5). The flow range covered by the sampling events was 111,496 to 184,703 m^3 day^{-1}, representing low to medium flow (Qmean = 166,422 m^3 day^{-1}; **Figure E-1**). During each sampling event, a 5-L wastewater sample was collected from each of eight sample collection points (P): influent before screens (P1), preliminary effluent after coarse screening and grit removal (P2), primary effluent phase 1 (P3a) and phase 2 (P3b), secondary effluent phase 1 (P4a) and phase 2 (P4b), secondary effluent mixed liquor (P5), and final effluent after tertiary treatment (P6) (**Figure 5-1**). Samples were collected in the morning, with two additional afternoon samples on the same day during SE5 from the influent (P1, pm) and effluent (P6, pm), to explore daily fluctuations. A bulk sample, taken by lowering a metal bucket into the stream, was filtered through a 2.8 mm metal sieve, and collected in plastic bottles for transport to the laboratory. Bottles were kept in black plastic bags at 3°C until processing within a maximum of 8 weeks after collection.

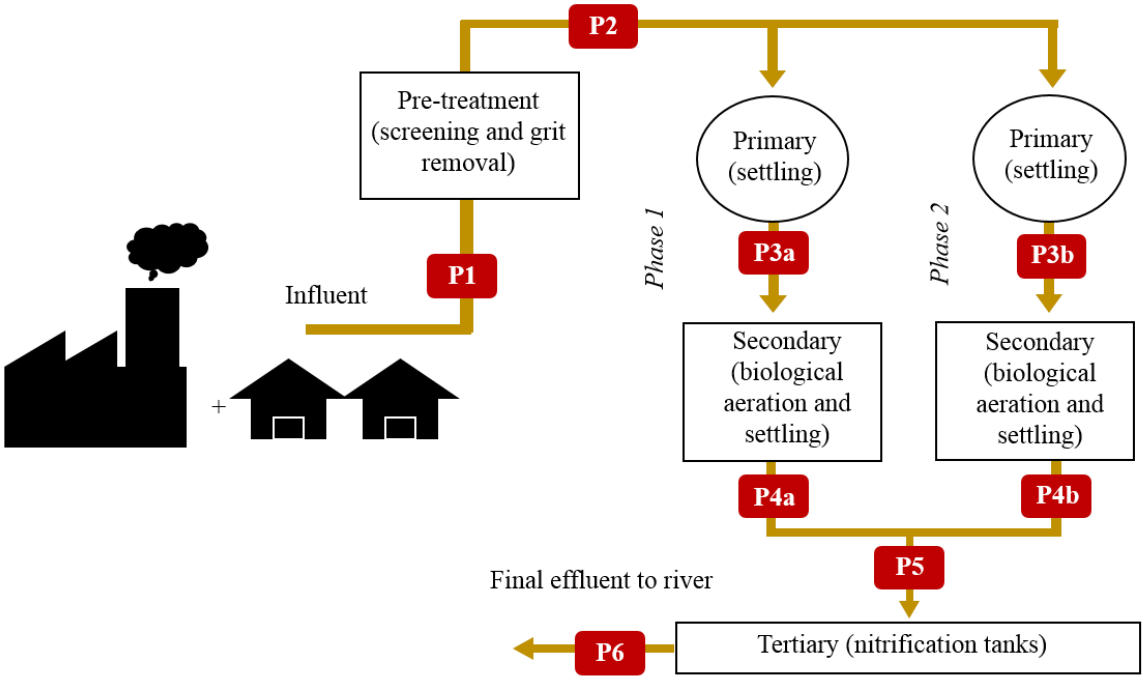


Figure 5-1 Generalised diagram of the tertiary sewage treatment process in the selected study site, illustrating eight sample collection points (P1-P6).

5.2.2 Microplastic extraction

The methodology for extraction and characterisation is broadly adapted from wet peroxide oxidation (WPO) protocols (Nuelle et al. 2014). As sewage can contain pathogens, all samples were processed in a Category 2 biological safety cabinet (Cat 2 BSC) and room, which also helped minimise potential background contamination of samples. Samples were transferred to glass Erlenmeyer flasks and spiked with 50 standard polyethylene (PE) beads each (0.71-0.85 mm diameter, ρ=0.96 g cm^{-3}; Cospheric LLC, Santa Barbara, California), to determine recovery rates. The spiked samples were treated with 30% hydrogen peroxide (H_2O_2; 1:1, v/v) for digestion of labile organics, heated in a water bath to 75°C for 30 minutes to accelerate the reaction, stirred using a magnetic stirrer for 10 minutes, and digested at room temperature for three days. After the digestion period, samples were treated with UV light for 30 minutes to ensure they were sufficiently sterile to be removed from the Cat 2 BSC room for filtration under vacuum through Whatman 1.2-µm glass fibre filters (47 mm diameter). This processing stage was very time-consuming, indeed samples still contained some level of suspended solids and therefore filtration of 5-L samples was slow and required several filters. It was the step that limited the volume of samples that could be processed between sampling events. However, the entire sample was processed and filtered in this fashion to minimise the potential loss of smaller MPs by on-site filtration.

5.2.3 MP characterisation

Particle characterisation followed a two-step process starting with visual sorting of suspected MPs into four categories based on morphology: pellets, fibres, fragments, and films. Each entire filter area was examined using a Leica MX_{75} stereo microscope with magnification between 10x and 32x to identify and quantify particles of size range between 60 and 2800 µm (Blair et al. 2019).

A subsample of 70 pieces, equivalent to 5% of total particles identified during visual inspection, was selected for chemical confirmation of plastics by Fourier-transform infrared-attenuated total reflectance spectroscopy (FTIR-ATR), using a Shimadzu IRAffinity-1S FTIR with diamond crystal and 20 scans. Manipulation of small particles was difficult, thus chemical analysis was only possible for fibres (n=19), fragments (n=10) and films (n=41) larger than 300 µm. Pellets could not be analysed as they were lost during transfer due to their small sizes and smooth surfaces. Materials were identified by comparing the unknown spectra to those in the Shimadzu LabSolutions IR libraries, which contain approximately 12,000 reference spectra. For each particle, the top three automated matches were compared visually to assess closeness of match, and except for four pieces, the highest score was considered acceptable and reported (**Table E-3**). The counts for confirmed plastics were used to estimate percentages for each category, subsequently extrapolated to correct all visual counts, including the 60-300 µm fraction. Further details of the FTIR-ATR characterisation process are in **Appendix E (Figure E-4, Table E-3)**.

5.2.4 Quality control

A procedural blank was created for each SE by running 5 L of DI water through the same sample equipment used to collect samples, and processed the same way as wastewater. The purpose of the procedural blanks was to evaluate possible cross-contamination from generation of particles from plastic equipment used during sampling – these include plastic bottles, synthetic ropes, and a plastic funnel. Laboratory blanks were created in triplicates by placing 1 L of DI water in the same glass containers used for sample processing and leaving uncovered on lab benches during the extraction process, and filtering in parallel with each run of field samples. The purpose of the lab blanks was to capture cross-contamination from deposition of airborne particles in the general environment. Procedural and lab blanks, respectively, contained 4-14 and 0-3 coloured fibres by count (**Table E-1**), while no other type of particles were observed. It was not possible to analyse fibres in the blanks chemically, but their presence is considered evidence of cross-contamination from the environment and the use of synthetic sampling ropes.

Fragmentation tests using MP-spiked DI water were carried out to assess if the extraction process could generate secondary MPs at various stages. This is reported in **Appendix F (Figure E-3, Table E-2**. It was found fragmentation could occur, but the MPs used to assess this (microbeads) were rare in the samples, and so this understanding could not be used to refine MP estimates.

5.2.5 MP estimation

For each category, visual counts were corrected by subtracting the corresponding procedural blank. To ensure MPs were quantified correctly, blank-correct data were multiplied by the percentage of FTIR-confirmed plastics in each category. Such FTIR correction was employed for conservative estimates of daily discharge from a secondary WWTP in Vancouver, although blank correction was not incorporated in their calculation (Gies et al. 2018). The FTIR-corrected counts were summed to estimate total MP abundance (items L^{-1}), for each stage and each sampling campaign. Daily flow data for the WWTP were used to estimate incoming and outgoing MP loads in items day^{-1} and stage-wise removal efficiencies.

5.3 Results and discussion

5.3.1 Chemical confirmation of MPs

During visual characterisation, a total of 1308 items across all samples were considered potential MPs: 871 fibres, 191 fragments, 239 films, and 7 pellets (n=7) (**Figure 5-2**). Chemical characterisation confirmed that MPs were present and comprised 39% of the total pieces measured by FTIR-ATR (**Figure 5-3**). Within each category of suspected MPs, plastics comprised 63%, 80%, and 17% of fibres, fragments, and films respectively. In absence of chemical confirmation and thus based on appearance, all micropellets (the lowest abundance of particle) recovered from wastewater samples were counted as primary MPs. Thus, based on FTIR-corrected data, a total of 749 MPs were observed across all wastewater samples, consisting of 549 fibres, 153 fragments, 41 films, and 7 pellets.

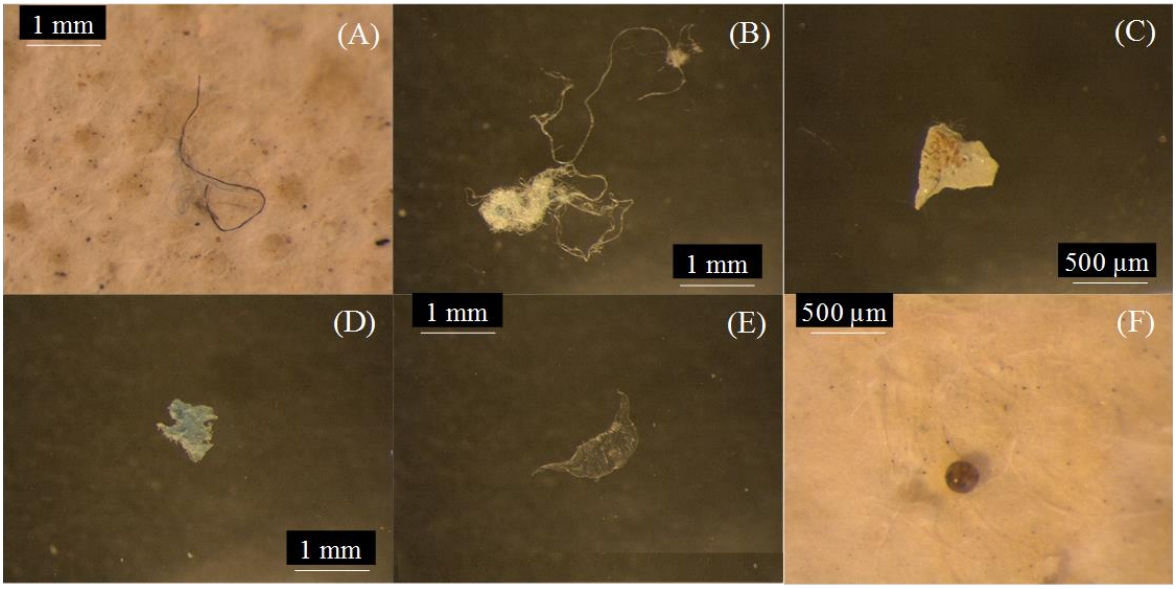


Figure 5-2 Examples of secondary and primary types of MPs extracted from wastewater samples and identified visually: fibres (A-B), fragments (C-D), film (E), and pellet (F)

Different types of polymers identified (**Figure 5-3**) included commonly-used plastics like polypropylene (PP, 23%) and PE (4%), and some less common, such as polyvinyl stearate (PVS, 7%) and polyoxymethylene (POM, 1%). The remaining MPs identified here were grouped as copolymers and included an ethylene-ethyl acrylate film and a PE-PP fragment. Polypropylene and PE are often reported in relatively high abundances across available surveys (Sun et al. 2019), as they are used in a wide number of applications including personal care and packaging products. The second-most detected polymer was PVS, a material not yet reported in other studies to date, and of limited use in the plastics industry (Gooch 2011). Polynivyl stearate can be co-polymerised with polyvinyl chloride, PVC (Gooch 2011) so may indicate construction applications. The POM particles also may not be common, only reported to date from a Danish secondary WWTP. The same study found PE-PP copolymers in raw and treated wastewater (Simon et al. 2018), but in higher abundance than this study.

Non-plastic materials were also present in the subsample (**Figure 5-3**): cellulose (36%), lecithin (13%), and protein (1%). While these are not the focus of this paper, their presence should still be noted as depending on sample purification process, they may not be entirely removed from samples and thus mistaken as MPs. The remaining pieces classed as “Other” included 5 fibres, 2 fragments, and 1 film. These particles could not be identified as they showed no distinguishable peaks to allow for manual annotation or to produce any hits during the library search (**Figure F-4**).

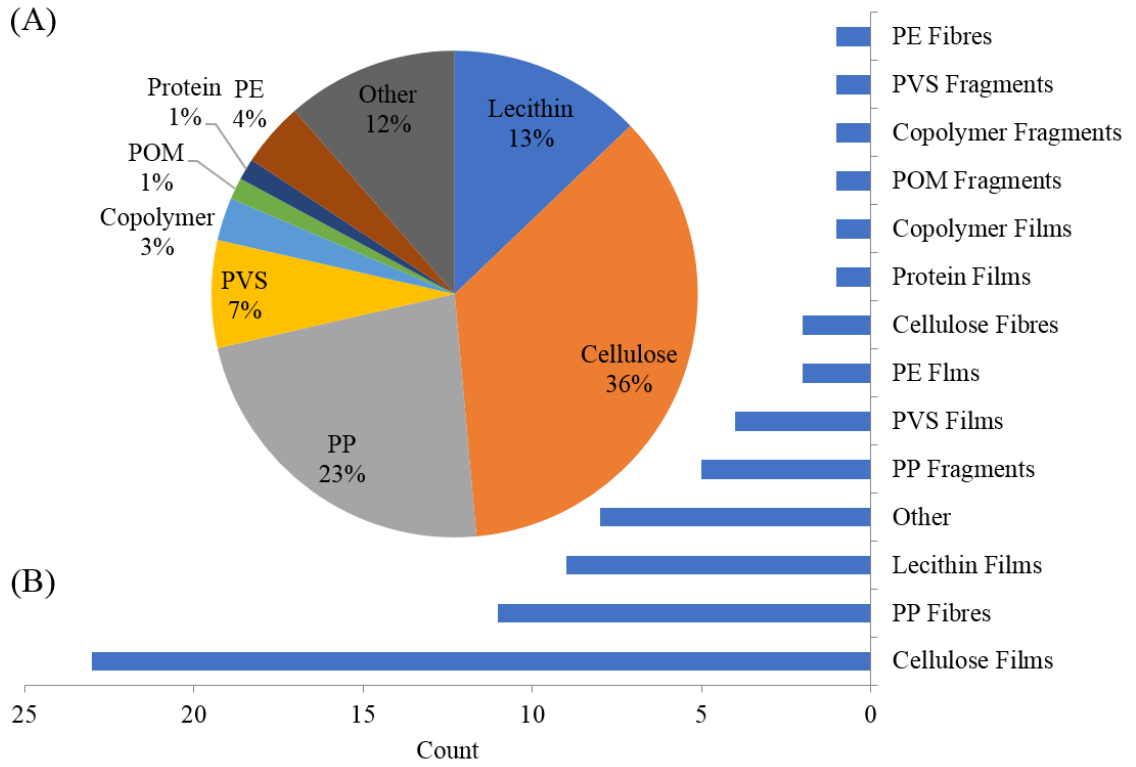


Figure 5-3 (A) Pie chart showing the chemical distribution in percentages of different types of materials identified in a subsample of suspected secondary MPs (n=70); (B) Bar graph showing the repartition by count for the chemical and categorical data combined.

5.3.2 MP morphology

Secondary MPs were predominant in the wastewater samples, comprising 99.5% of total pieces. Fibres were the most common type of MPs, followed by fragments and films. The predominance of fibres here is consistent with previous wastewater surveys (e.g. Sutton et al. 2016; Gies et al. 2018; Lares et al. 2018; Conley et al. 2019). Fibre abundance is expected to be higher in densely-populated areas as they can be carried by washing machine effluent. For example, clothes washing can release between 1.9×10^3 (Browne et al 2011) and 6×10^6 fibres per wash (De Falco et al. 2018). The highest releases have been observed from polyester (Pest) and polyamide (PA) garments, but these materials were not identified by FTIR-ATR here. This may be as Pest and PA fibres were settling out of suspension due to higher densities. Therefore, their concentrations in the liquid fractions would be lower than the detection limit allowed by a 5-L sample. Alternatively, they may have been smaller than 300 µm and thus were not subsampled for chemical identification. However, PP fibres may highlight the importance of other sources like sanitary products, thermal clothing, medical applications, and construction materials (Mandal 2019), but the discussion on these alternative sources of fibres to WWTPs is limited in the literature. Fibre count was highly-variable across sampling events, and while generally decreased after each treatment stage (**Figure 5-4**), some fibres persisted through the process and were observed in final effluent.

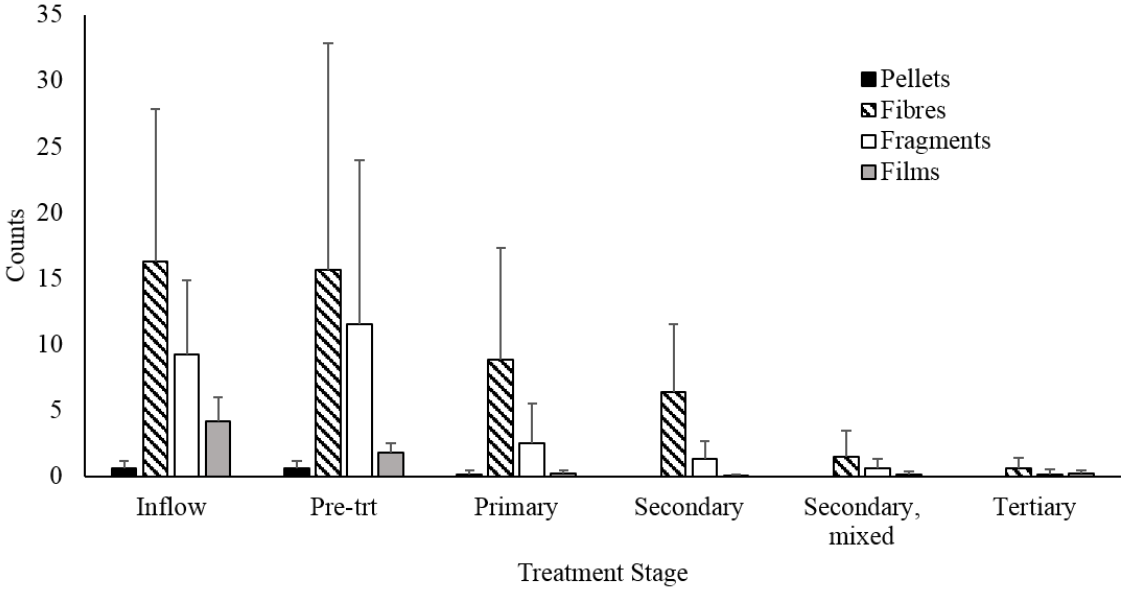


Figure 5-4 Mean counts of MPs at different stages using FTIR- and blank-corrected data calculated averaging all sampling campaigns. Error bars represent standard deviation.

Fragments were present throughout all treatment stages and at least one particle was observed in final effluent (**Figure 5-4**). Most fragment removal seemed to occur after the primary stage (when settling of solids takes place) and again after tertiary treatment. Films were mostly removed during pre-treatment, which may indicate they are more likely to be captured in the grit and grease biosolids as observed in a similar study in a Scottish secondary WWTP (Murphy et al. 2016). Different types of fragmented pieces have also been observed across multiple WWTPs (Sun et al. 2019) and generally refer to uneven or irregular pieces. As observed here, fragments were the second most-abundant MPs after fibres in a Swedish secondary WWTP (Magnusson and Noren 2014), in secondary and tertiary WWTPs in the USA (Mason et al. 2016; Sutton et al. 2016), and in an Italian tertiary treatment plant (Magni et al. 2019). Here, fragmented pieces were categorised as either films or fragments to distinguish between two-dimensional thin particles and three-dimensional pieces with broken edges, respectively. However, the terms used to categorise these particles may vary across surveys (Hidalgo-Ruz et al. 2012), thus it is necessary to unify classifications for adequate consideration.

Fragments can be produced from a wide variety of sources and enter the wastewater stream via household and industrial effluent, but fragments generated during the treatment process cannot be excluded, supported by evidence of fragmentation of larger MPs beads (>700 µm) in controlled tests here. This needs to be validated for other particle types and sizes. Furthermore, the WWTP may have plastic equipment that if degrades over time could release MPs, but to our knowledge this has not been explored. The mechanical generation of MP fragments, particularly in sizes that may be evading detection, presents an important research gap in these systems that warrants further investigation as without it WWTP loading and MP redistribution cannot be fully understood.

Lastly, microbeads were only observed before secondary treatment (**Figure 5-4**). This is consistent with previous observations in Swedish secondary WWTPs where 95-99% of microbeads were considered to settle out in sludge (Magnusson and Noren, 2014), and in the UK where microbeads

were only found in grease fractions removed during pre-treatment (Murphy et al. 2016). These observations are for particles >65 µm. Therefore, entrapment in sludge may explain why these particles were only observed in the early treatment stages in this study also. Primary MPs (i.e. microbeads) can be introduced to WWTPs via household sewage, but primary MPs represent only a small portion of the plastic load in this catchment. This discussion is relevant to current considerations on MP control measures of MPs, especially as current actions such as regulatory bans are mainly aimed at reducing primary MPs inputs, and few focus on secondary sources.

5.3.3 MP abundances

Microplastics were present throughout the system. Concentrations ranged from ~1 to 13 MPs L^{-1}, with highest abundances in pre-treatment effluent during SE1 (**Figure 5-5**). Total concentrations of MPs were highly-variable across sampling dates and time, consistent with other reports of high variability (Sun et al. 2019). Influent concentrations were between 3 and 10 MPs L^{-1}, with maximum abundances observed in January and minimum in February and July. In effluent, concentrations were between <1 and 3 MPs L^{-1}.The lowest concentrations were mostly observed after tertiary treatment (final effluent), except during SE2, when concentrations reached their minimum after the mixed secondary liquor. Both influent and effluent abundances observed here are comparable to those in a secondary WWTP in Glasgow, Scotland (Murphy et al. 2016) but considerably lower than in three secondary WWTPs in South Carolina, USA (Conley et al. 2019). Nevertheless, current methods may not be suited to detect small MPs (e.g. <300 µm) so it is probable that MP concentrations are underestimated, especially as small MPs have been observed in greater abundances than larger pieces (Carr et al. 2016; Mintenig et al. 2017). Moreover, small MPs (e.g. 20-190 µm) may be more common in final effluent as they are more likely to pass through filtration barriers if not retained in biosolid fractions and smaller than the pore size (Ziajahromi et al. 2017; Sun et al. 2019).

Abundances were highly variable across sampling events and between the morning and afternoon samples collected on the same day, despite similar flow conditions. A survey of three USA WWTPs observed concentrations to vary by a factor of 2.5 and 4.8 in influent and effluent respectively, and long-term variations were greater than in short-term (Conley et al. 2019). However, the absence of replicates in the present study limited this assessment of short-term variation, and future work should explore this to support considerations of regulating inflow concentrations of different types of MPs to the system.

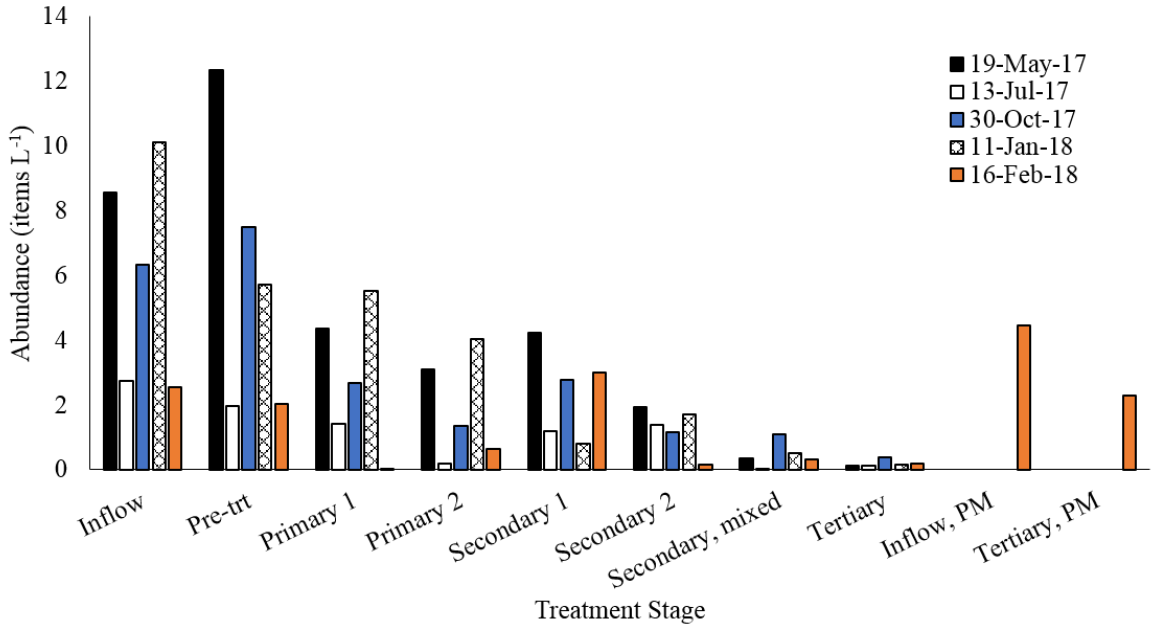


Figure 5-5 FTIR-corrected MP abundances across all treatment stages and events in a tertiary sewage treatment plant.

5.3.4 MP removal and loadings

Average MP inflow to the treatment plant over one year was 8.1 x 10^8, 95% CI [3.8 x 10^8, 1.2 x 10^9] particles day^{-1}. Influent loads based on incoming concentrations and plant flows are only reported by a few studies (Magnusson and Noren 2014; Murphy et al. 2016; Lares et al. 2018; Conley et al. 2019), but their findings suggest these loads may be partially dependent on the size of population served. For example, among three WWTPs in South Carolina, a WWTP serving 1.8 x 10^5 p.e. received considerably higher MP loading than a treatment plant serving a smaller population (Conley et al. 2019). In an earlier survey in the same catchment of this study in Scotland, a larger secondary treatment plant serving 6.5 x 10^5 p.e. received an average daily load of 4 x10^9 MPs >65 µm. Incoming loads in the present study were mostly comparable to those of a Finnish secondary treatment plant (p.e. not specified) in Finland with a reported daily inflow of 6.2 x 10^8 MPs >0.25 µm (Lares et al. 2018).

Particles concentration decreased between influent and final outflow with each treatment stage removing different proportions of MPs (**Figure 5-6**). Mean concentrations decreased by 6% (standard error 16) after pre-treatment. Preliminary treatment has only been assessed by two studies, and removal efficiencies in this research are lower than those reported, ~35-58% (Michielssen et al. 2016; Murphy et al. 2016). Primary treatment removed between 60 (P3a, standard error 10) and 76% (P3b, standard error 6) of overall MP counts and is consistent with other surveys (63-81%, Dris et al. 2016; 84-88%, Michielssen et al. 2016; 78%, Murphy et al. 2016; 97.4-98.4%, Talvitie et al. 2017b; ~68%, Ziahjaromi et al. 2017). There was indication of further removal after secondary treatment, but this was only evident at the secondary mixed liquor stage after the channels are joined back together (P5). As there is no remediation between P4 and P5 stages, this reduction suggests that engineering parameters and infrastructure may play a role in MP retention, especially if a large

portion of removal is attributed to settling. After secondary treatment (P5), removal reached 92% (standard error 3), comparable to a Finnish secondary treatment plant where 7-20% of MPs were removed by activated sludge treatment (Talvitie et al. 2017b). A similar study in a larger UK secondary treatment plant had a retention efficiency of 98% and discharged 6.5 x 10^7 particles day^{-1} (Murphy et al. 2016). Although the data come from different WWTPs, both studies are located in the same catchment, serve a similar population demographic, and observed a similar profile of MPs. Therefore, the differences between the two plants emphasise that removal of MPs will depend on site-specific engineering parameters besides loading and general treatment process.

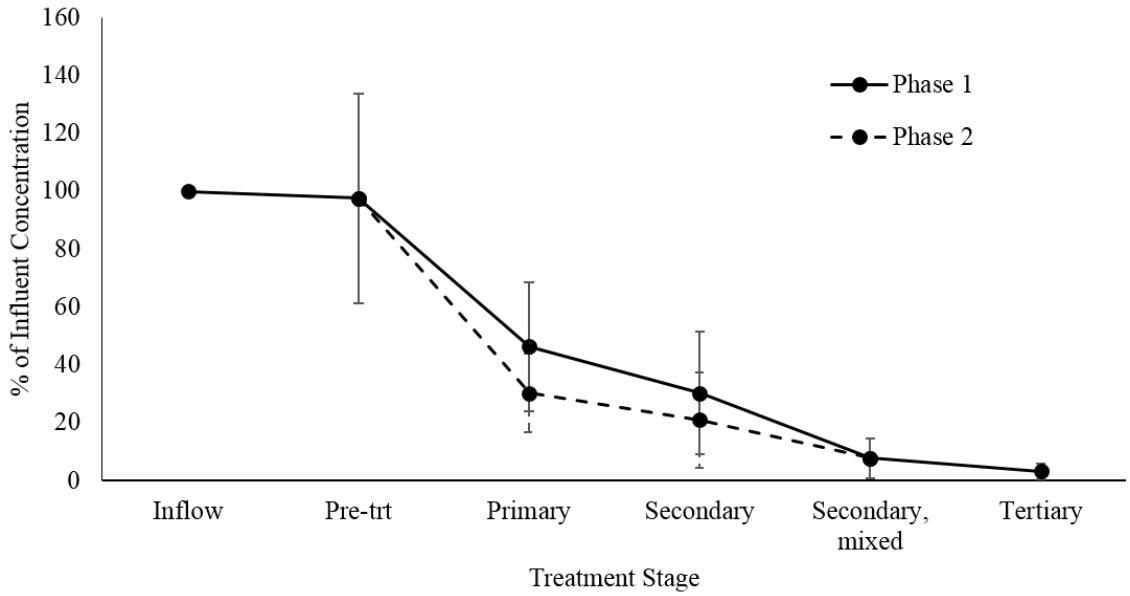


Figure 5-6 Percent change relative to influent microplastic concentrations after each treatment stage, averaged across five sampling campaigns. Concentrations are FTIR- and field blank-corrected, then averaged across the five sampling events.

Tertiary treatment produced an average 4% (standard error 1) decrease in MPs in secondary effluent, bringing the total retention efficiency to ~96% (**Figure 5-6**). The plant discharges on average 2.2 x 10^7, 95% CI [1.2 x 10^7, 3.2 x 10^7] MPs day^{-1} under low- to medium-flow conditions. The removal ranges and discharges here are within those observed elsewhere (**Table 5-1**), noting cross study comparisons are difficult as different sampling volumes and size ranges can introduce uncertainty to MP measurements reported across sites. No other sites of the same type of treatment considered here (i.e. use of plastic media in nitrifying trickling filters) have been documented, but removal percentages in this WWTP were higher than those observed by advanced sand filters (Magni et al. 2019) and lower than MBR (Michielssen et al. 2016; Talvitie et al. 2017b). The differences among these treatment technologies may be expected because of differences in the porosity of the filters they use, and so may indicate a way in which performance of tertiary treatment may be predicted. Nevertheless, the diversity of advanced systems and the contrasting results reported for different facilities, mean more research in WWTPs is needed to help identify which technologies optimise removal of MPs pollution in and from these systems.

5.4 Conclusions

Here, the occurrence, distribution, and fate of MPs in an advanced WWTP were assessed. A continuous input of MPs and other microdebris to the treatment site was observed over the course of ten months. The presence of MPs was confirmed by FTIR-ATR analysis, with PP identified as the most abundant type and present as fibres and fragments. Microplastics were mainly observed as secondary types, and while a few pellets were present, their chemical composition could not be determined due to size limitations of the FTIR-ATR approach employed here. Fibres were dominant. Their high abundance is expected as they are often associated with washing machine effluent, but their presence in blanks suggests that some may be entering the system via atmospheric, possible as the wastewater is treated in open channels. The system investigated here had apparent removal efficiencies at the higher end of that observed elsewhere, but MPs were not entirely removed and at least 1.2×10^7 particles may be discharged daily from this site even during low flow. These estimates are limited to particles sized 60-2800 µm but there will be smaller MPs in the system that need to be investigated further. As observed by other studies, the largest concentration reduction was observed in early treatment stages. Generally, this is linked to retention of microplastics in the sludge and so the concentration and fate of MPs in sludge needs further attention because rather than providing a solution, it may be displacing delivery of MPs to the environment. This research generates new understanding of MPs in WWTPs by its consideration of multiple stages, including tertiary treatment, not yet considered elsewhere and by employing a longer sampling period in a single facility to generate spatio-temporal understanding. Further research could use larger sample volumes to reduce the blank sensitivity and incorporate greater sampling frequency to assess short-term variation and thus contextualise seasonal observations. As wastewater treatment plants are expected to play an increasingly important role in regulating the delivery of MPs coming from land-based sources, this and similar studies can help to inform regulators about what needs to be prioritized in monitoring programmes and where controls should be implemented, thus guiding fundamental action.

5.5 Addendum

Here, additional information is presented for colour distribution of MPs observed in Daldowie wastewater and MP abundances in activated sludge samples from the secondary WWTP Shieldall, which discharges into the Clyde Estuary. These data are included because they are relevant to understanding sources and fate of MPs in anthropogenic and natural water systems.

Colour characterisation of MPs along with other visual and chemical properties will produce distinctive combinations and thus can be of high value to infer source of origin. Furthermore, the combined shape, colour, and chemical analysis can be used to determine if MPs in samples and those in background contamination are from the same source – this will help to improve the use of quality assurance tests in data correction. Additionally, although ecotoxicity was not investigated here, colour may play a role in the fate of MPs, as different MPs may be ingested by different animals based on colour (Lusher et al. 2013; Provencher et al. 2017).

Analysis of the Daldowie activated sludge contributed to understanding where MP may partition in WWTPs, as it was not possible to analyse this fraction from Daldowie. Quantifying MPs in this

sludge fraction is important because while a portion of activated sludge may be removed with primary sludge, some is returned back to the system and so this provides insight on MP recirculation in the treatment process. Additionally, as microorganisms are mixed with wastewater in the activated sludge process, it is important to ensure a healthy microbial community is maintained, but it is unknown if MPs present in this sludge fraction could threaten these microorganisms. For example, leaching of bisphenol A from PVC in lab experimentation has been observed to have a negative effect on microorganisms involved in anaerobic digestion of waste activated sludge (Wei et al. 2019).

5.5.1 Colour repartition

Coloured pieces at Daldowie WWTP were more common than pale MPs when data were pooled together across all sampling stages and events (**Figure 5-7**), but the categorical repartition data showed that these results were largely driven by the relative abundance of fibrous MPs. While coloured fibres comprised 87% of total fibre count, pellets and films were mostly non-coloured (86% and 78% respectively), and pale and coloured fragments were equally abundant (49% and 51%, respectively) (**Figure 5-7c-f**).

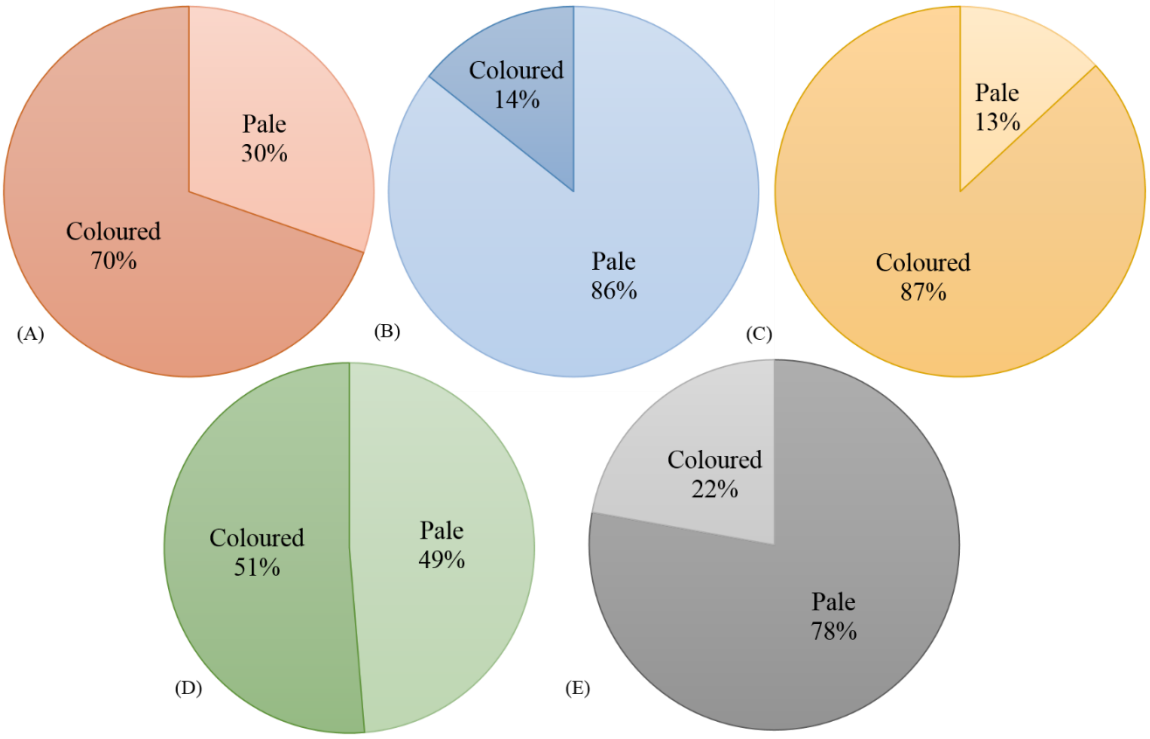


Figure 5-7 Colour distribution for all visually-identified pieces across all sampling stages and events (A, n=1308) and for each category: pellets (B, n=7), fibres (C, n=871), fragments (D, n=191), and films (E, n=239). Primary, beads or spheres; secondary, fibres, fragments, and films; pale, clear or white pieces; coloured, non-clear/white pieces.

Particle colour is only reported by ~50% of the wastewater studies, but can be important and thus should be used as part of visual assessment. In wastewater studies, colour data can be combined with

other evidence such as polymer shape for identification of sources of origin. For example, in southern California, USA, blue MPs were determined to resemble particles originating from whitening toothpaste (Carr et al. 2016), while clear PE fragments recovered from Finnish WWTPs were associated with cleaning scrubs (Talvitie et al. 2017b; Lares et al. 2018). This type of assessment is important in understanding site-specific loadings and identifying and regulating MP inputs. Furthermore, there is some evidence that plastic colour may be an indicator of exposure to chemicals like PCBs and plastic additives (Provencher et al. 2017).

Colour is also an important consideration for standardisation of guidelines for visual identification (Provencher et al. 2017). Transparent and green pieces may be common in nature while bright colours like orange, red and blue are more characteristic of anthropogenic particles (Dris et al. 2015). However, as certain colours may be more eye-catching depending on the background (e.g. colour of filter paper), this may introduce a potential source of analytical error that should be considered when interpreting results to ensure that certain types of MPs are not under- or overestimated (Hidalgo Ruz et al., 2012; Cole et al., 2014; Murphy et al. 2016; Lares et al. 2018). For chemical characterisation, FTIR analysis may be more difficult for colourless and black pieces because these may require alternative accessories for their adequate examination. (Leslie et al. 2017).

5.5.2 Activated sludge

It is thought that decrease in MP concentrations in liquid fractions of WWTPs is associated with retention of these particles in sludge fractions. While MP concentration in biosolids was not assessed for Daldowie, different types of MPs (**Figure 5-8**) were observed in two activated sludge samples from Shieldhall WWTP, with concentrations of 7 and 12 items L^{-1} (**Table 5-2**). The categorical distribution of MPs was similar to that observed in wastewater from Daldowie, with fibres more common, followed by fragmented pieces and finally pellets. Chemical characterisation for MPs in the Shieldhall samples was only possible by SEM-EDS due to a lack of access to FTIR-ATR equipment at the time the samples were processed. The elemental characterisation by SEM-EDS was used to distinguish MP pellets, fragments and films from non-MPs. While MP fibres could not be differentiated chemically from cellulose, SEM-EDS allowed for enumeration of two additional fibres and one film that were not observed during visual identification (**Table 5-2**).

Table 5-2 Counts and abundances of MPs in two activated sludge samples from Shieldhall WWTP determined by visual characterisation under light microscopy and elemental analysis by SEM-EDS.

Identification	Sample	Microplastic count (n)					Abundance (items L^{-1})
		Primary	Secondary			Total	
		Pellets	Fibres	Fragments	Films		
Visual	1	6	4	9	4	23	23
	2	2	3	8	0	13	13
Chemical	1	1	4	2	5	12	12
	2	0	5	2	0	7	7

Figure 5-8 Example of MPs observed in activated sludge samples from Shieldhall WWTP, including a clear pellet (top left), fibre cluster (top right), yellow honeycomb fragment (bottom left), and clear film (bottom right).

The presence of MPs in the activated sludge samples here supports the interpretation that MPs may be immobilised by various sludge fractions (e.g. Carr et al. 2016, Murphy et al. 2016; Lares et al. 2018; Li et al. 2018). However, studies considering sludge fractions are limited (Sun et al. 2018) and of those available only two studies have sampled activated sludge. Reported concentrations in these two studies range from 5 x 10^1 particles kg^{-1} (size not specified) in a US WWTP to 2.3 x 10^4 particles kg^{-1} (sized >250 µm) in a Finnish WWTP. In activated sludge systems, some of the excess sludge is removed with the sewage sludge fraction, while a portion of the activated sludge is cycled back into the system, thus MPs retained in this fraction may not entirely be removed from the WWTP. Furthermore, activated sludge contains important microorganisms needed for breakdown of organic material. Therefore, contaminants associated with the microplastics that could poison the bacterial communities are undesirable. There is a research need for the direct (components added to the plastic) and indirect (chemicals adsorbed by the plastic) effects of MPs on the microbial component of WWTPs to be explored.

Despite removing the majority of incoming MPs, the WWTP may still discharge considerable amounts of MPs to the environment on a daily basis, mainly fibres. Therefore, the last part of the research was to investigate spatio-temporal MP distribution in the recipient river, up- and downstream from the WWTP to assess if there is an influence of WWTP discharge on MP abundance in the river.

6 Microplastics in the freshwater recipient channel of a tertiary WWTP: distribution, sources, and retention and release

Abstract

Microplastics have been observed in freshwater rivers worldwide. These MPs may be introduced to the riverine environments from diffuse sources such as urban runoff, as well as point sources like WWTPs, as evidenced in the previous chapter. Rivers are important pathways for transport of MPs from land to sea, but the empirical data for these aquatic systems remains limited. The research conducted in the River Kelvin provided evidence that MPs may be retained in sediments, while studies elsewhere observed transport of MPs by lotic waters indicating that retention may not be permanent. As MPs differ in type, size, and densities, their entrapment and transport may differ by their association with different catchment parameters like land and water use, hydrodynamics, and sedimentation processes. Therefore, the last part of the research summarised in this chapter aims to investigate the sources, retention and release of MPs by assessing their spatiotemporal distribution and characteristics in a large river, the River Clyde, which is exposed to numerous point- and non-point inputs of anthropogenic pollution. The possible association between MPs and water parameters and sediment size distribution were explored. Microplastics were present in bank sediment and surface water sampled between August 2018 and June 2019. Microplastics were observed in sediment up-and downstream from the WWTP discharge, indicating contributions from both diffuse and point sources. Concentrations in sediment were 1-26 items kg^{-1}, and consisted primarily of clear PET and PP fibres resembling fishing lines. Microplastics in surface water were only observed downstream from the WWTP, with a maximum of 4 MPs 24 L^{-1} and were mainly in the form of PP and PE fragments. As sampling was conducted during low to medium flow conditions, the presence of MPs in water suggests that release to and by rivers is continuous and is not limited only to periods of high rainfall. For the freshwater portion of the River Clyde and the period of study evaluated, the maximum concentration could represent a transport of up to 6 x 10^8 MPs at medium flow. The majority of MPs were associated with the 1.0-0.3 mm sediment size fraction, regardless of type, and there was no relationship between MPs and water quality. Understanding of the sources and retention and release of MPs by riverine systems is needed to design integrated catchment management strategies for remediation of MP pollution.

6.1 Introduction

Despite the recent advancements in freshwater river studies, the intra- and inter-study variability in MP concentrations observed across sites, and the methodological challenges means that there is still limited understanding of sources, transport and fate of these contaminants in riverine systems. Rivers are crucial links between anthropogenic MP sources to their delivery and accumulation in oceans so warrant further attention to develop mitigation strategies at the catchment level. Rivers can receive MP pollution from point and non-point sources as rivers drain a wide range of catchments, so their study can provide information to trace source of origin. Furthermore, the study of spatiotemporal distribution patterns can further understanding on the effect of hydrodynamic and environmental factors on movement of different types of MPs depending on size, shape, and density. This information can provide insight on whether MPs follow similar or different pathways as other contaminants and their dependency on sediment transport dynamics. When not transported to oceans, studying the behaviour of MPs in rivers can also further understanding on the retention in sediments, biofouling or transferral across the land-water interface. Therefore, a large freshwater river, the River Clyde was sampled to advance understanding of entrapment and transport of MPs to and by the river.

The River Clyde receives freshwater inputs from various tributaries and direct discharges from the tertiary WWTP described in the previous empirical chapters. As it drains urban areas and becomes the Clyde Estuary, it is particularly relevant for understanding the contribution of river transport to MPs pollution in marine systems. Furthermore, larger rivers carry more contaminants (Galgani et al. 2000), and those closer to populated areas and receiving sewage discharges may have more advanced pollution (Kataoka et al. 2019), so studying the River Clyde can provide more insight into the distribution and transport of particles, complementing some of the initial findings obtained from its smaller tributary, the River Kelvin. The specific objectives for this part of the PhD project were to: (1) investigate the characteristics (chemical and physical) and spatiotemporal abundance of MPs, (2) consider the potential contributions of MPs from the WWTP and other diffuse and point sources of pollution, and (3) assess the potential retention and release of different types of MPs.

6.2 Sampling sites

Collection sites in the River Clyde were selected as described in section 3.1.3 based on location relative to point and non-point sources of pollution, and constrained by accessibility to the river and sediment deposition zones. Sampling was conducted at five stations in an urban freshwater stretch of the river before the tidal weir, with two sites upstream (UP) and three downstream (DO) from the WWTP discharge pipe. Five sampling sites were selected as more spatial samples, while aspirational, were not possible due to time constraints in processing the samples whilst incorporating replicates. While the two upstream sites were selected as representative of sites without immediate WWTP discharge, they are still exposed to point-source pollution from CSOs. Moreover, there may be other WWTPs discharging further upstream from the selected study location. Furthermore, both upstream and downstream sites are subject to various diffuse sources of anthropogenic debris from the confluence of tributaries and surface runoff from industrial, residential, and recreational activities along the catchment (**Figure 6-1; Table 6-1**). Additionally, all sites are exposed to atmospheric deposition to the open stream and likely to pollution from fishing and recreational activities.

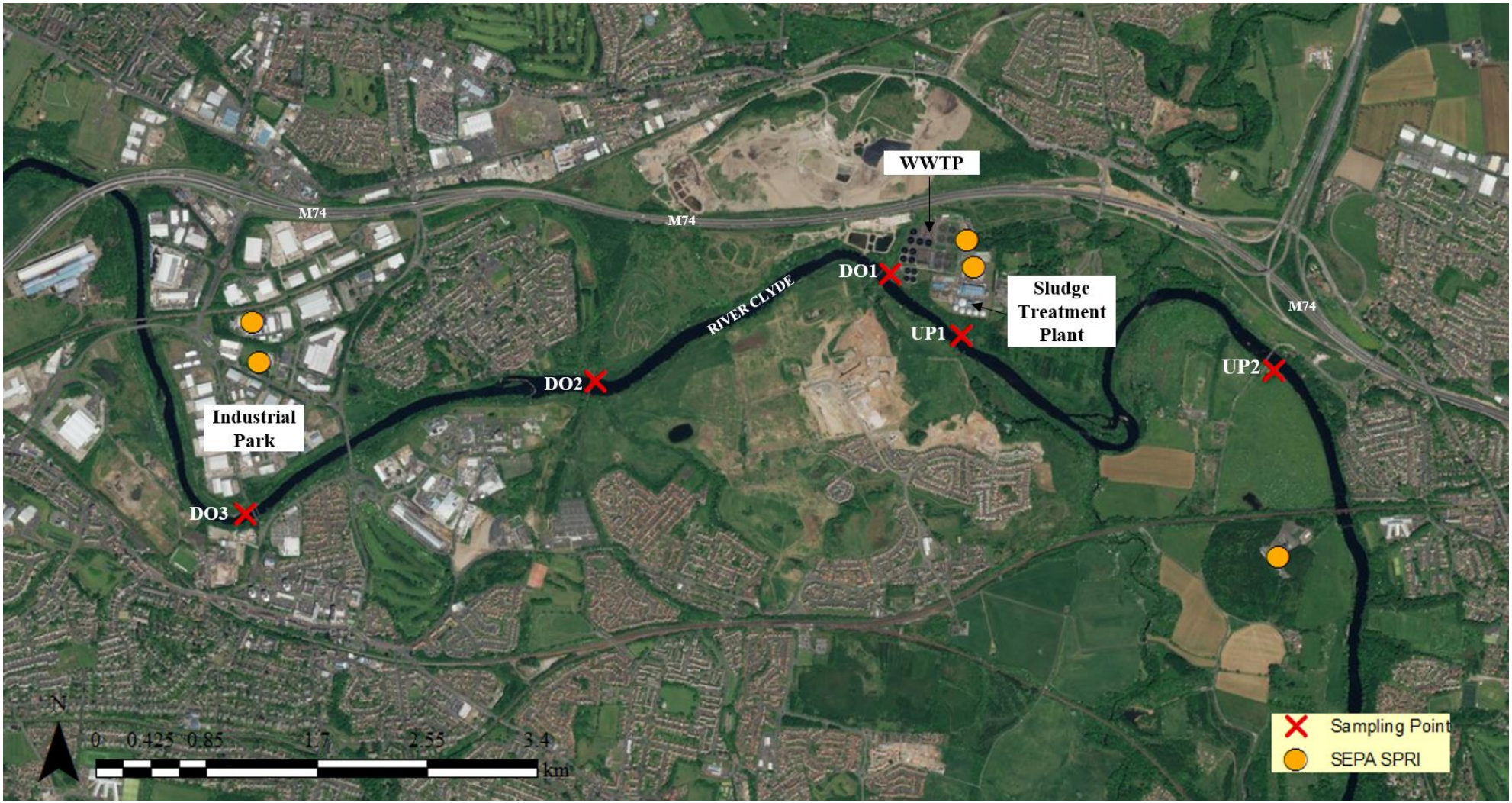


Figure 6-1 Location of five sampling points in the Upper River Clyde study. **WWTP**, wastewater treatment plant; **DO**, downstream of WWTP; **UP**, upstream of WWTP; **SPRI**, Scottish pollution release inventory. SPRI data copyright © SEPA. ArcGIS online basemap copyright © Esri. All rights reserved.

Table 6-1 Description of sampling stations in the River Clyde, relative to their location upstream (UP) and downstream (DO) from Daldowie WWTP

Site ID	Sample	Distance from WWTP (km)	Potential inputs of anthropogenic debris	
UP2	Sediment Surface Water	2.91	Major highway on the opposite bank that is separated from the river channel by a vegetation buffer; discharge from CSO pipe; road bridge.	
UP1	Sediment	0.53	Confluence of two tributaries; road bridge; Crematorium grounds; new construction of residential area (aerial photograph)	

Table 6-1continued Description of sampling stations in the River Clyde, relative to their location upstream (UP) and downstream (DO) from Daldowie WWTP

Site ID	Sample	Distance from WWTP (km)	Potential inputs of anthropogenic debris	
DO1	Sediment	0.07	WWTP discharge pipe; sludge treatment plant discharge pipe; daily activities from both treatment sites (photograph looking upstream towards discharge pipe)	
DO2	Sediment	2.36	Confluence of small burn; residential area; recreational activities in cycling/pedestrian path along river channel	

Table 6-1continued Description of sampling stations in the River Clyde, relative to their location upstream (UP) and downstream (DO) from Daldowie WWTP

Site ID	Sample	Distance from WWTP (km)	Potential inputs of anthropogenic debris	
DO3	Sediment Surface Water	5.12	Surface runoff from industrial estate; two CSO discharge pipes; recreational activities in cycling/pedestrian bridge; residential area and road on opposite bank	

6.3 Modifications to sampling and extraction protocols

Sampling and extraction of bank sediment and surface water in the river followed sediment and wastewater protocols used in the previous experiments with some modifications (sections 4.2 and 5.2).

6.3.1 Sample collection

Sediment and water samples were collected in the morning and in duplicates during each of three sampling campaigns between August 2018 and June 2019. Sediments were sampled in duplicates at all five sites in August 2 and 23, and November 7, 2018. At each site except UP1, submerged sediment samples were collected at the water line where the bank is exposed during low flow, using a spade to scoop up the sediment. At UP1, access to the site is via a gauging station with steps leading directly into the channel and no visible exposed or submerged sediment, even during low flow. Therefore, at this station, a telescopic rod with detachable plastic beaker was submerged into the channel to collect bottom sediment as close as possible to the river-bank. Access to the UP1 site was not possible during the third visit, so this site was sampled only during August. All sediment samples were collected in aluminium trays and covered with tin foil for transport back to the lab for immediate processing.

Due to logistical constraints of collecting and processing a larger number of samples, surface water samples were collected only at two sites representing the upstream and downstream points furthest from the WWTP (**Table 6-1**). First, plankton net sampling (500 mm frame, 63 µm mesh) was attempted but due to site conditions and time limitations, it was not possible to continue with this approach. Here, the focus was on achieving a cut off size of 63 µm comparable to the sediment fractionation protocol, thus allowing for a broader inspection of MP pollution than those reported in most studies. Therefore, it was decided to use an approach similar to the wastewater sampling by lowering a bucket into the stream to collect a 24-L composite sample. The water sample was pre-filtered on site through stacked Tyler sieves with mesh 2.8 mm and 63 µm, after the size range employed for sediment fractionation. The coarser debris retained in the 2.8 mm sieve was discarded, while material in the 63 µm was rinsed with DI water and collected in glass jars for transport back to the lab for immediate processing. While water sample volumes are not always mentioned in the literature, studies using net sampling report varying times of deployment (e.g. from a few minutes to several hours) that depend on how quickly the filters are clogged by drifting organic debris and could result in highly-variable volumes. The bucket approach allowed for controlled collection of a consistent sample volume for improved comparability across sites and replicates. Water samples from each site were collected in duplicates three times: February 21, April 9, and June 3, 2019. At DO3, samples were collected from the middle of the nearest pedestrian bridge. At UP2, samples were collected from a non-functioning SEPA gauging station near the river bank because of lack of access to the centre of the river.

6.3.2 MP extraction and quantification

The sediment samples were processed as described for the River Kelvin protocols in section 4.2, with slight adjustments. First, sediment samples were oven-dried at 80°C instead of the 100°C used in the Kelvin experiment. While 100°C is at the threshold of melting point for common plastics and not expected to alter the materials, a lower temperature was selected for the final protocol to improve consistency with previous methods as most report temperatures between 60-80°C for this step (**Table A-2**). Furthermore, the higher temperature of 80°C was selected instead of 60°C to reduce processing times since a lower temperature would require samples to be in the oven for a longer period. For each sample, approximately 500 g of oven-dried sediment were weighed and size-fractioned using a Tyler sieve shaking apparatus into the following classes: <2.8 mm, 1.0 mm, 0.355 mm, 0.18 mm, and <0.063 mm. Fewer size classes were employed here compared to the Kelvin protocol as based on Kelvin data, it was reasonable to pool some intermediate size fractions. Doing so, improved comparability with recommended methods (Masura et al. 2015) and allowed incorporation of spatial sampling and replicates since it reduced processing times. The separate sediment size classes were treated with NaCl density separation followed by double-settling, as described in section 4.2.3. Water samples were extracted without any prior treatment as the use of the 2.8 mm sieve allowed for removal of coarser organics in the field and suspended sediment was not an issue. This allowed processing of the entire sample in a similar fashion to the wastewater protocol and reduced the potential loss of MP particles from additional extraction steps. All samples, including the NaCl sediment extracts and untreated surface water were filtered under vacuum through a 1.2 um GF filter in a similar way to the wastewater samples. Filters were placed in petri dishes and dried at 60°C to remove excess water from filters and prepare for identification.

Similarly to the Kelvin and Daldowie research, visual identification with a stereo microscope was the first step of identification and enumeration. Representative aliquots comprising 15% and 56% of total pieces detected visually in sediment and water samples, respectively, were examined by FTIR-ATR and used to correct the visual counts, as described in section 5.2.5 for wastewater protocols. As various particles were unidentifiable in the Daldowie study due to high noise in the signal, 40 scans were used for this study to reduce the baseline noise and allow for more distinguishable peaks.

6.3.3 Contamination controls

Precautions were taken to minimise contamination during sampling and processing, such as keeping samples covered, and using glass, metal, and aluminium equipment instead of plastics, where possible. Spiking of samples and lab controls were also employed in this experiment to explore background contamination and particle recovery. The PE beads and PP fibre-like standards described in section 3.3 (**Figures C-2 and C-3**) were used for spiking controls, while open containers with water were placed near the work benches and filtered in parallel with each run as in the wastewater protocol.

Laboratory blanks extracted during sediment runs contained between 0 and 3 microfibres with mean 1 ± 0.8 and a total of 16 fibres observed across 14 blank runs (**Table F-1**). Most of these fibres were dark-coloured and short (**Figure F-1**) with only 3 white or pale fibres. Background contamination

for water extraction runs (n=3) contained 0, 3 and 9 fibres. Since water samples were filtered with no prior treatment, these blanks were only exposed to atmospheric deposition for a short time, suggesting that fallout of airborne particles can occur very rapidly. It was not possible to obtain chemical confirmation of blank fibres. Some of these particles were collected from the filters with the aid of the stereo microscope, but as the FTIR-ATR used here was not equipped with a microscope, the particles were lost during transfer for chemical analysis.

Atmospheric fallout was not measured in the field, but visual images and FTIR-ATR spectra were produced for synthetic ropes used during water sample collection for comparing with fibres in the samples. Except for one, all pale fibres in water samples were determined visually and chemically similar to the sampling ropes (**Figure F-2**). As MPs can be produced by mechanical breakdown, it is likely that these fibres were released due to the rubbing of the rope with the bridge railings while pulling the sample up for collection. The quantity of fibres released by this sampling approach was unknown, but all fibres visually and chemically resembling rope fibre standards were eliminated from the final counts to account for this potential cross contamination.

Particle recovery in spiked controls ranged from 60 to 100% for both beads and fibres across 17 lab blanks (**Table 6-2**). This indicates that even when there are no other organic or inorganic materials in suspension, particle recovery can be reduced during extraction. As laboratory blanks were created with DI water, particle loss here may be attributed to the affinity of MPs to adhere to the extraction containers. Additional validation tests were conducted by spiking sediment and water samples with PE beads and PP fibres. Sediment samples collected at DO2 and UP2 during the second campaign were used for spiking. All six surface water samples were spiked before filtration. Recovery of spiked MPs from sediment was lower than from DI blanks, ranging from 20 to 90% and UP2 showing comparably higher recovery than DO2 (**Table 6-2**). The lower recovery from sediment is likely due to loss of MPs during decanting of the supernatant as some MPs may be retained in the container with the settled solids. Furthermore, the lower recovery in DO2 may be because samples collected from this site contained more organic material than UP2 that may entrap MPs, but this assessment is only visual (**Figures G-1 and G-2**) as OM content was not quantified. Particle recovery from water was comparable to that in DI standards between 60 and 100% for beads, and 80 and 100% for fibres. Where visible, spiked particles attached to the container or sediment were removed with tweezers. Even so, full recovery was not always possible highlighting challenges in current extraction methods. To address these challenges, it may be necessary to incorporate sample spiking and report particle recovery as common practice in routine testing.

Table 6-2 Particle recovery in laboratory blanks and downstream (DO) and upstream (UP) sediment and water samples spiked each with 10 PE bead and 10 PP fibre standards per extraction run. **NA**, not available; **a**, replicate 1; **b**, replicate 2.

Date	Sample ID	Recovery (%)	
		PE Beads	PP Fibre
	- Lab blanks -		
02-Aug-18	Blank Run 1	80	80
	Blank Run 2	70	80
	Blank Run 3	80	80
	Blank Run 4	80	100
	Blank Run 5	80	100
23-Aug-18	Blank Run 1	90	100
	Blank Run 2	90	80
	Blank Run 3	100	90
	Blank Run 4	100	100
	Blank Run 5	100	70
07-Nov-18	Blank Run 1	70	100
	Blank Run 2	70	70
	Blank Run 3	100	100
	Blank Run 4	NA	NA
	Blank Run 5	60	90
22-Jan-19	Blank Run 1	70	100
09-Apr-19	Blank Run 1	90	70
03-Jun-19	Blank Run 1	60	90
	- Spiked samples -		
23-Aug-18	DO2a spiked	30	60
	DO2b spiked	20	20
	UP2a spiked	50	80
	UP2b spiked	60	70
22-Jan-19	DO3a	90	100
	DO3b	100	100
	UP2a	90	80
	UP2b	70	100
09-Apr-19	DO3a	90	80
	DO3b	80	90
	UP2a	90	90
	UP2b	80	100
03-Jun-19	DO3a	100	90
	DO3b	80	80
	UP2a	60	80
	UP2b	90	90

6.3.4 Water quality

Water quality parameters were monitored throughout all sampling campaigns to evaluate the pollution level of the river. Grab samples were collected from sites DO3 and UP2 during all sediment and water sampling campaigns (n=12), and from DO1, DO2, and UP1 during sediment sampling events only (n=4-6). Samples were collected in duplicate from the same location of sediment collection, and kept in 1-L HDPE bottles in 3°C until analysed. Samples were analysed following standard protocols for determination of pH and electrical conductivity (EC; µS cm^{-1}), and concentration (mg L^{-1}) of total organic C (TOC), total carbon (TC), inorganic carbon (IC), nitrate (NO_3) + nitrite (NO_2), and sulphate (SO_4). Briefly, pH and EC were measured from unfiltered water samples with an ISFET portable pH meter and a VWR EC300 portable conductivity meter, respectively. Total organic C, TC, and IC were determined from unfiltered samples, using a Shimadzu TOC-L equipped with an ASI-L autosampler. Lastly, the ion chromatography (ICS-900, Dionex, USA) technique was used to measure NO_3 + NO_2, and SO_4 following filtration of ~10 mL aliquots of each sample through a 0.45 µm Nylon syringe filter.

Analysis of variance (ANOVA) was performed using the *Agricolae* package in R (SAS Institute, Cary, NC) to determine significant differences in water parameters across stations. Least significant difference (LSD) and a critical value of $p < 0.05$ were used for means separation and comparisons. Water parameters were consistent throughout the study area, except for higher NO_3+NO_2 concentrations at DO1, immediately after effluent discharge (**Table 6-3**). There was no correlation between MP abundance and water parameters (**Figure H-1**).

6.3.5 River Discharge

River discharge data from Daldowie gauging station (at UP1) were provided by SEPA. River discharge during sampling events ranged from 20 to 42 m^3 s^{-1}, representing medium flow conditions for the catchment for the period of study (**Figure 6-2**). The highest flows sampled were during November (sediment) and January (water) and were close to the mean river discharge of 40 m^3 s^{-1} for the sampling period from August 2018 to June 2019. The remaining sampling events captured flows in the 50-70th percentile thus approaching low flow conditions.

Table 6-3 Water pH, electrical conductivity (EC), nitrate + nitrite ($NO_3 + NO_2$), sulphate (SO_4), total organic carbon (TOC), total carbon (TC), and inorganic carbon (IC), measured at five sites during sediment sampling events (1-3; n=6 unless otherwise specified) and at two sites during all sampling events (1-6; n=12). Least significant difference (LSD) test and a critical value of $p < 0.05$ were used for means separation. **UP**, upstream, **DO**, downstream

Site	pH	EC (µS cm^{-1})	$NO_3 + NO_2$	SO_4	TOC	TC	IC
			mg L^{-1}				
			*Events 1-3**				
UP2	7.45[a]	322.82[a]	4.77[b]	19.71[a]	9.67[a]	28.2[a]	18.53[a]
UP1(n=4)	7.57[a]	386.23[a]	10.73[b]	48.22[a]	10.27[a]	32.17[a]	21.9[a]
DO1(n=5)	7.33[a]	408.12[a]	29.51[a]	44.48[a]	8.9[a]	27.84[a]	18.94[a]
DO2	7.36[a]	336.28a	12.63[b]	39.77[a]	9.32[a]	28.91[a]	19.59[a]
DO3	7.08[a]	366.07a	9.92[b]	41.23[a]	10.42[a]	31.85[a]	21.43[a]
p-value	0.24	0.10	0.00	0.36	0.95	0.41	0.69
			Events 1-6				
UP2	7.40	303.40	4.86	17.79	11.68	26.65	14.97
DO3	7.22	270.39	8.62	30.24	12.16	29.34	17.18
p-value	0.05	0.42	0.06	0.08	0.83	0.29	0.52
LSD	0.18	84.15	3.88	14.13	4.56	5.12	6.96

* LSD not available because *n* is not consistent for all sites

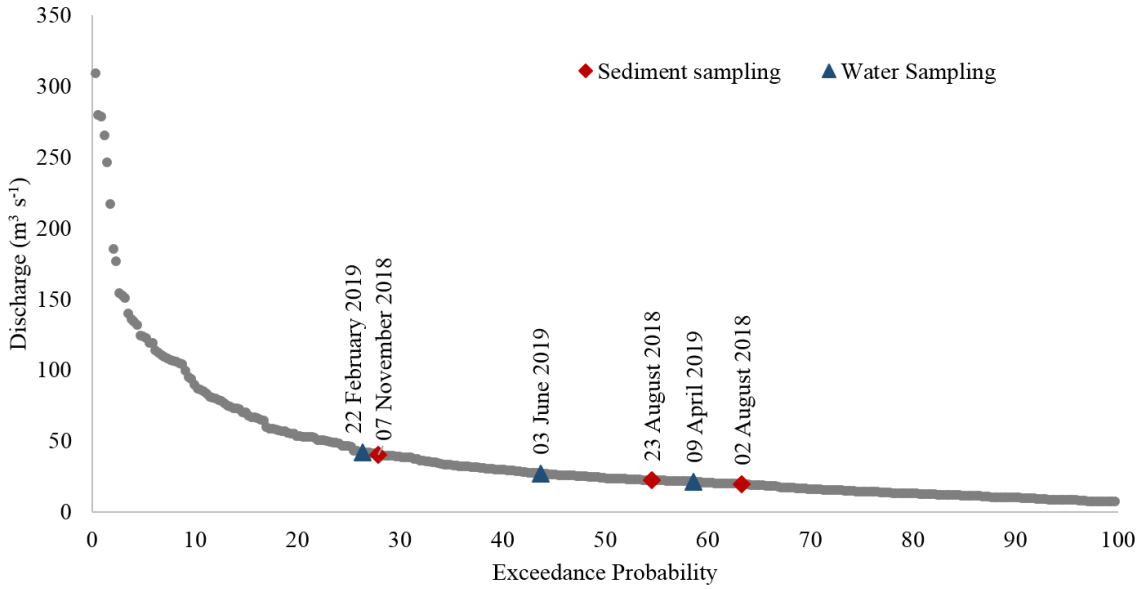


Figure 6-2 Flow duration curve for the Clyde catchment for the period of study from August 2018 to June 2019. Contains SEPA data from Daldowie gauging station © Scottish Environment Protection Agency and database right 2019. All rights reserved.

6.4 Results and discussion

6.4.1 Chemical composition

A total of 241 suspected MPs were counted across 27 sediment samples collected from five stations. Of these suspected MPs, 35 particles were subsampled for FTIR-ATR analysis: 3 pellets, 10 fibres, 12 fragments, and 10 films. Approximately 46% of the pieces were identified as synthetic polymers (**Table 6-4**) including commonly-used thermoplastics like PET/Pest and PP. Similar to this study, these polymers were listed as the most common plastics in sediments of the River Thames (Horton et al. 2016), and as they have a wide number of applications, their presence was expected. The polymer composition of analysed specimens varied by category. Only one pellet was identified as a clear PP bead, and while a sphere classified as unknown could be plastic, in absence of chemical confirmation it was excluded from final counts (**Table 6-4**). Fibres were predominantly plastic, mainly PET/Pest. Microplastic fragments included PP, PA, and PET/Pest all common plastics. Additionally, other less common plastic materials included PBT, a vinyl chloride/vinyl acetate/vinyl alcohol terpolymer and a butyl methacrylate/isobutyl methacrylate copolymer. While PBT has not been mentioned in previous studies, it is a common polymer used in electrical and electronic applications belonging to the same family of Pest (omnexus.specialchem.com 2019). Considering that copolymers derived from two or more types of monomers, were also identified in wastewater, the presence of a co- and terpolymer here could reflect future tendencies towards their increasing abundance in the environment as they can offer opportunities to create a wide range of new plastic products (Wang et al. 2017b). Therefore, these materials may be of increasing interest to policy makers.

A total of 45 pieces were observed across 12 water samples collected from two spatial points. It was possible to analyse more than half of the particles extracted from water samples for a total of 25 particles characterised chemically: 19 fibres and 6 fragments. The single film observed initially was lost during transfer and was dismissed from final counts. Most FTIR pieces were identified as common plastics including PP, PE, and PA. Of 16 fibres identified as PP, 15 were considered cross-contamination from the sampling rope. The remaining fibres were identified as PA or EVA copolymer, and one unknown. Fragments were mostly plastics composed of PP, PE, and PVC.

Particles composed of PE and PP are the most commonly-identified plastics across river surveys and were prevalent in surface waters of Swiss rivers (Faure et al. 2015), the Three Gorges Dam system (Zhang et al. 2015), Illinois and Indiana rivers in USA (McCormick et al. 2016), the Xiangxi River in China (Zhang et al. 2017), the Antua River in Portugal (Rodrigues et al. 2018), Teltow Canal in Berling (Schmidet et al. 2018), and 29 Japanese rivers (Kataoka et al. 2019). Their high abundance is attributed to their multiple uses in daily products, while EVA is often copolymerised with PE and so may reflect similar applications. The blue PP fibre was similar to those in wastewater suggesting a potential association between the two systems, while a single black fibre was the only identified PA material. The general absence of PA contrasts with general findings in the literature that suggest that PA along with Pest textiles have the higher release rates of fibres compared to other types of polymers and thus are abundant in environmental samples (DeFalco et al. 2018). However, as

discussed in Chapter 6, the absence of these materials from the samples could be attributed to limitations from the analytical method.

Table 6-4 Chemical composition by category of suspected MP pieces extracted from River Clyde sediment and water sampled between August 2018 and June 2019.

Material	Pellets	Fibres	Fragments	Films	Total
		Sediment			
Terpolymer	0	0	0	1	1
PET	0	6	0	0	6
Pest	0	2	1	0	3
PP	1	1	1	0	3
PA	0	0	1	0	1
PBT	0	0	0	1	1
Copolymer	0	0	1	0	1
Total Plastics	1	9	4	2	16
Kaolin	1	0	1	1	3
Tencel	0	0	2	1	3
Nitrocellulose	0	0	0	1	1
Reshicin	0	0	0	1	1
Glass	0	1	0	0	1
Cotton	0	0	0	1	1
Lithium Carbonate	0	0	1	1	2
Protein	0	0	3	0	3
Sediment	0	0	0	1	1
Unknown	1	0	1	1	3
Total Non-Plastic	2	1	8	8	19
TOTAL SEDIMENT	3	10	12	10	35
		Water			
PP	0	16	1	0	17
PE	0	0	2	0	2
PA	0	1	0	0	1
EVA	0	1	0	0	1
DEHP/PVC	0	0	1	0	1
Total Plastics	0	18	4	0	22
Tencel	0	0	1	0	1
Epoxy Resin	0	0	1	0	1
Unknown	0	1	0	0	1
Total Non-Plastic	0	1	2	0	3
TOTAL WATER	0	19	6	0	25

Non-plastic materials were also present in the sediment and water subsets selected for FTIR. Non-plastic microdebris comprised 46% of the total pieces analysed. Lecithin films that were identified in the wastewater samples were also observed in river sediment, while cellulose-based materials like nitrocellulose, Tencel, and cotton were also present (**Table 6-4**). Some pieces deriving from naturally-occurring materials like clay (Kaolin) and sediments grains were visually misidentified as MPs in the samples during the first step of characterisation. Four pieces consisting of a pellet, a fragment, and a film could not be identified as the spectral match scores were below the established acceptance level of 700. Non-plastics were less common in surface water, comprising 12% of pieces analysed. The non-plastics in surface water were identified as Tencel and epoxy resin fragments, and one fibre of unknown origin. As these materials are not the main focus of this research project, they are noted as they can be misidentified as MPs during visual inspection and lead to overestimation, but are not discussed further.

6.4.2 MP morphology

Microplastics of different shapes, sizes, and colours were observed in sediment (**Figure 6-3**) and water (**Figure 6-4**) in the River Clyde. After FTIR correction, a total of 118 MPs were observed in sediment: 4 pellets (**Figure 6-3a**), 87 fibres (**Figure 6-3b**), 19 fragments (**Figure 6-3c**), and 8 films (**Figure 6-4d**). A total of 10 MPs were identified in surface water after FTIR confirmation, and consisted of two secondary types: 4 fragments (**Figure 6-4a-b**) and 6 fibres (**Figure 6-4c-f**).

Fibres were the dominant type of MP by count in sediment and water samples. Fibres were mostly pale or translucent and sediment, which contrasts to observations in the River Kelvin where coloured fibres were more abundant. Visually, these pieces resembled long lines with a consistent width and colour throughout their entire length and were observed in small knots or clusters. Conversely, coloured fibres dominated in surface water in the recipient river, similar to the liquid fraction in wastewater. Red and light blue fibres in river sediment and water were similar to some of the fibres observed across Daldowie wastewater samples, and based on visual comparison of photographs, they also had some resemblance to those observed in Great Lake tributaries (Baldwin et al. 2016) and the Three Gorges Reservoir system (Di and Wang et al. 2018), but this comparison with other studies is subjective.

Fragmented MPs and films were the next most abundant category. Fragments are often reported as a dominant category in freshwater river studies, although different categories are used to classify them, making direct comparability difficult. The presence of fragments observed in this study, however, likens to surveys in the North Shore Channel, USA (McCormick et al. 2014), Rivers Elbe, Mosel, Neckar and Rhine in Germany (Wagner et al. 2014), Lake Ontario tributaries (Ballent et al. 2016), the River Thames (Horton et al. 2016), the Antua River (Rodrigues et al. 2018), various Japanese rivers (Kataoka et al. 2019), and the Atoyac River in Mexico (Shuri et al. 2019). Fragments in this study were mostly coloured pieces of different shapes and sizes in both sediment and water samples.

Microplastic films and pellets were observed in sediment only. Films consisted of both opaque and light pieces, and were observed as rolled films and flattened sheets. Rolled films bore a resemblance to pieces observed in wastewater sampled before tertiary treatment in this study, and those observed

in sludge from a wastewater treatment plant in Spain (Bayo et al. 2016). Pellets were the least abundant type of MPs, which is consistent with findings from the River Kelvin and Daldowie and with the majority of freshwater river studies elsewhere. Pellets were generally small (e.g. <500 µm) and consisted of both dark and pale pieces. These characteristics are consistent with those of pellets observed in abundance in the River Rhine, where spheres were described mainly as translucent and opaque pieces < 1 mm (Klein et al. 2015; Leslie et al. 2015; Mani et al. 2015). However, yellow and blue spheres not present in the Clyde were also observed in one of the Rhine studies (Mani et al. 2015).

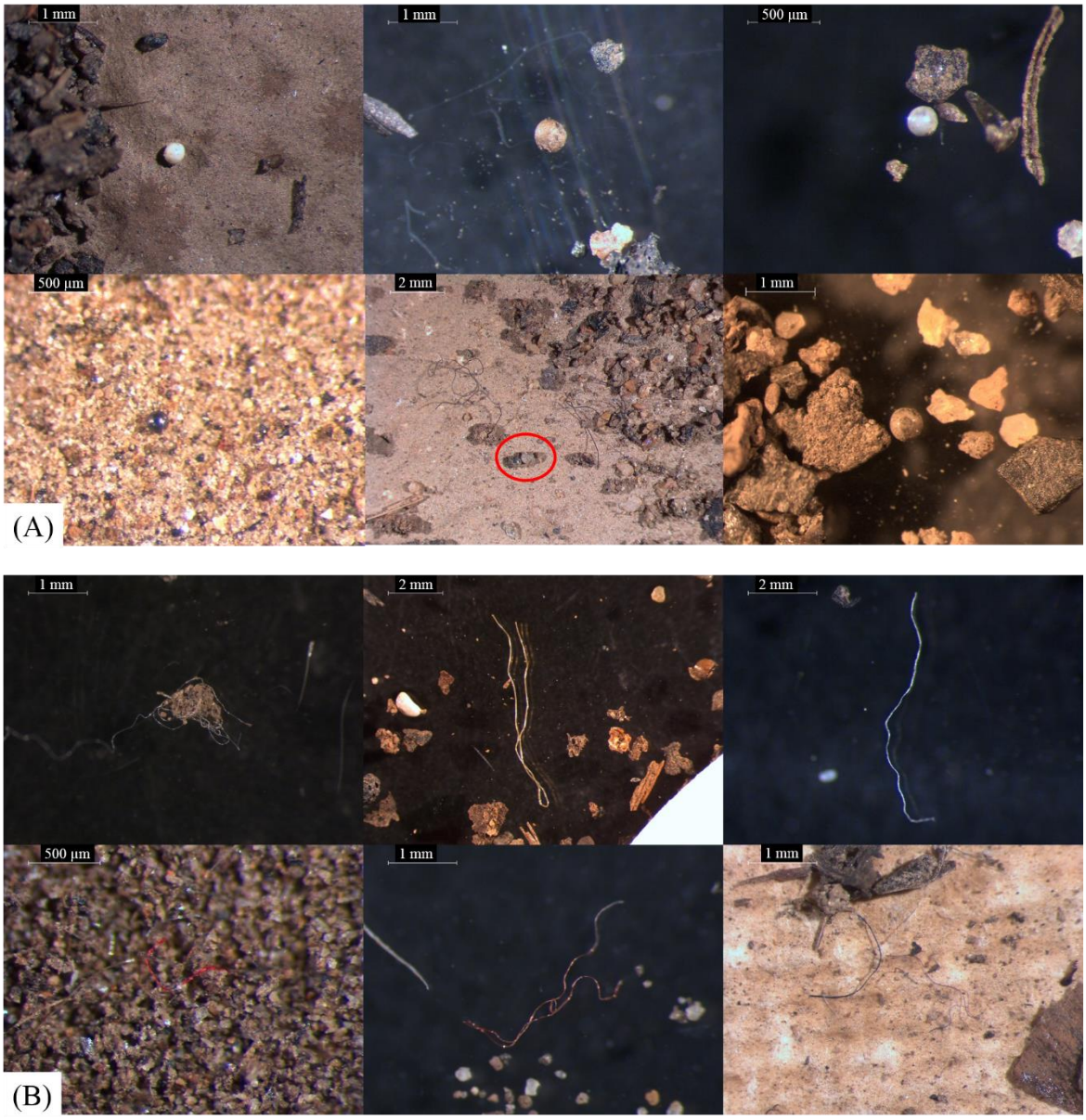


Figure 6-3 Examples of common suspected MP pellets (A), fibres (B), fragments (C), and films (D) extracted from River Clyde sediment sampled during August and November 2018.

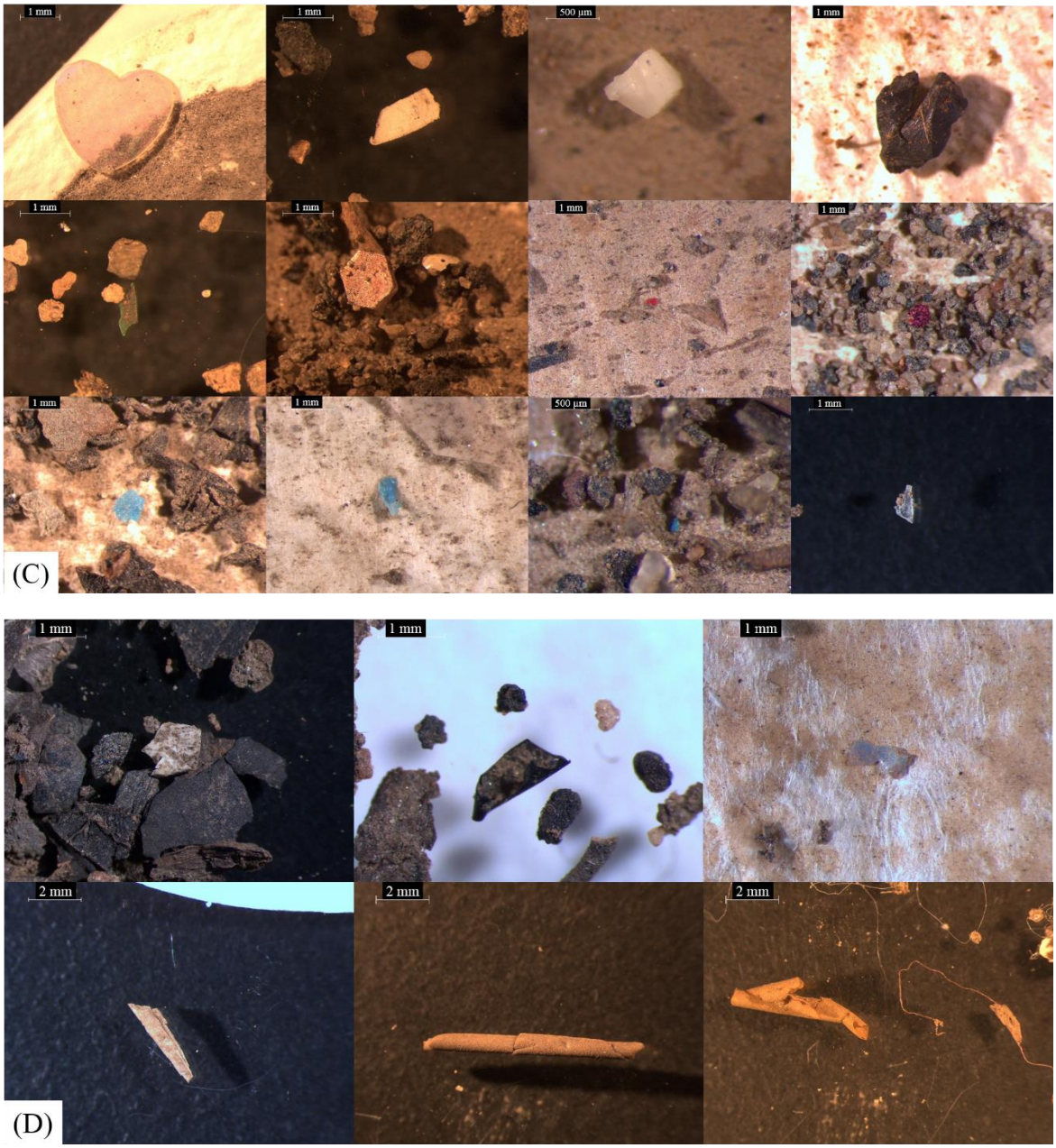


Figure 6-3 (continued) Examples of common suspected MP pellets (A), fibres (B), fragments (C), and films (D) extracted from River Clyde sediment sampled during August and November 2018.

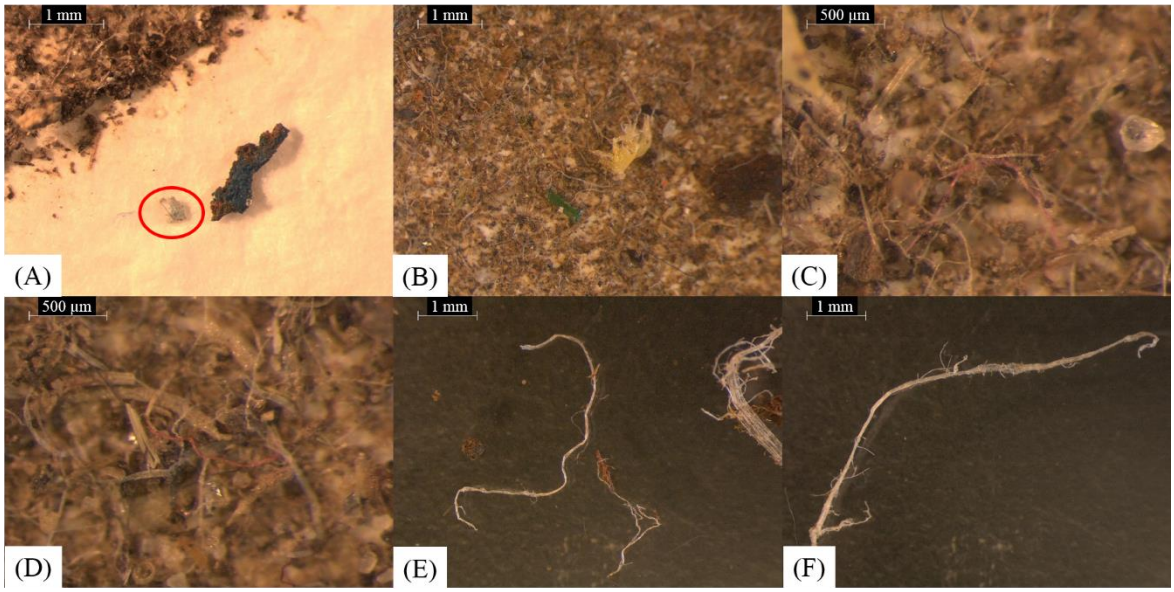


Figure 6-4 Examples of common suspected MPs fragments and fibres extracted from River Clyde water sampled during January, April, and June 2019.

6.4.3 Spatiotemporal abundance of MPs

Microplastics were present in both sediment and water samples in the River Clyde. Mean abundance in sediment samples was 9 ± 6 MPs kg^{-1} and ranged between 1 and 26 items kg^{-1}, with maximum concentration observed in DO3 during the November sampling event (**Figure 6-5**). These concentrations are lower than those observed in other studies that employ similar methods and comparable size ranges (Klein et al. 2015; Hurley et al. 2018; Rodrigues et al. 2018). High levels of MP pollution have been reported in the River Tame system, ranging from 100 to 6.2 x 10^4 items kg^{-1}. Higher MP concentrations have also been observed in river sediments of the Rhine and Main in Germany (228-3763 MPs kg^{-1}; Klein et al. 2015) and in the Antua River in Portugal (99-627 MPs kg^{-1}; Rodrigues et al. 2018). While comparability is limited by analytical methods, generally high abundances have been positively associated with population density and industrialisation of urban catchments (Ballent et al. 2016; Shruti et al. 2019). Therefore, the lower concentrations observed in this study may indicate that MP pollution is less advanced in this catchment and other factors such as geomorphology and seasonality may play a role in MP pollution levels. For example, a study in South Africa observed an abundance of 160 MPs kg^{-1} in the Bloukrans River during July, but a lower concentration of 6.3 MPs kg^{-1} in February, indicating seasonal variations (Nel et al. 2018).

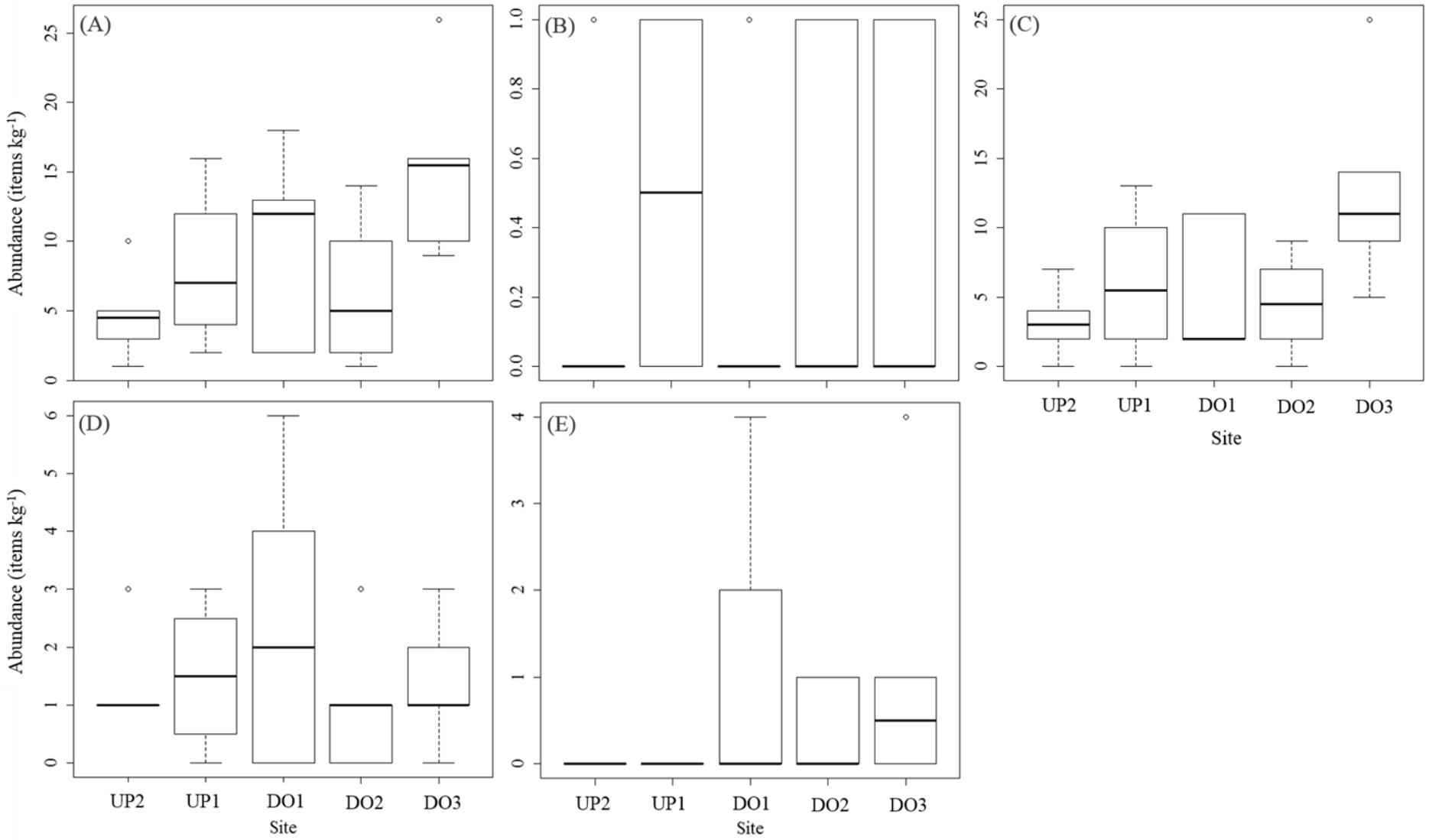


Figure 6-5 Microplastic abundances in spatial sediment samples from the River Clyde for all items (A) and by type: pellets (B), fibres (C), fragment (D), and films (E).

Total abundances averaged across all sediment sampling events (n=4-6) were significantly higher in UP2 compared to DO3 ($p<0.05$) but no differences were observed among sampling stations located between the two ends of the sampling stretch (**Figure 6-5**). This is consistent with increases in MP concentrations from upstream to downstream sites reported elsewhere, especially in systems receiving WWTP effluent (Browne et al 2011, Dubaish and Liebezit 2013; Estahbanati and Fahrenfeld 2016). Within a single sampling event, a slight decrease in MP abundance was observed from DO1 to DO2, followed by an increase at DO3. However, these observations were based on two replicates and when averaged across all sampling campaigns, the changes were not significant. Furthermore, a few differences were observed in the type of MPs observed across spatial points. Fibres were observed in significantly greater abundance in DO3 compared to UP2 when averaged for all campaigns ($p<0.05$), and were present along all spatial samples. Fragments were present in similar abundances across upstream and downstream sites with no significant differences across sites. Films were identified in downstream sites only, while pellets were predominant in downstream sites but were also observed at one upstream site.

Microplastics in surface water were only observed in the downstream sites and ranged between 0 and 4 MPs 24 L^{-1}, with maximum concentration roughly equal to 166 MPs m^{-3}. (**Figure 6-6**). Given that low to medium flows were captured during the period of study, when sediment deposition is more likely to occur due to reduced river discharge, these concentrations may reflect the low end of MP transport in this river. Higher concentrations in surface water may be expected during high rainfall events (Hurley et al. 2018; Nel et al. 2018). Therefore, further seasonal sampling in the Clyde is needed to be conclusive on the extent of MP pollution. As most surface water studies elsewhere use 300-333 µm net sampling and different sampling volumes, it is not possible to compare the results from this study directly with others. However, studies in freshwater rivers in China (Wang et al. 2017b; Di and Wang 2018) using a pump sampler with mesh size 48-50 µm reported higher concentrations than those in the Clyde. Additionally, there is a possibility that concentrations in surface waters were underestimated by the size range considered. As smaller MPs are perceived to be more abundant in the environment than larger fractions, it would be expected that lowering the minimum limit of detection would result in higher concentrations. Conversely, studies in Chinese (Wang et al. 2017b) and Portuguese (Rodrigues et al. 2018) rivers observed that MPs > 300 um were more abundant in surface waters. Therefore, the cut-off size of 2.8 mm employed here may underestimate large MPs as these may be present in low concentrations and thus require sampling volumes larger than the 24 L used here. While the lower limit of detection is often cited as the main limitation for inter-study comparability, the maximum cut-off may also affect interpretation and comparison of results.

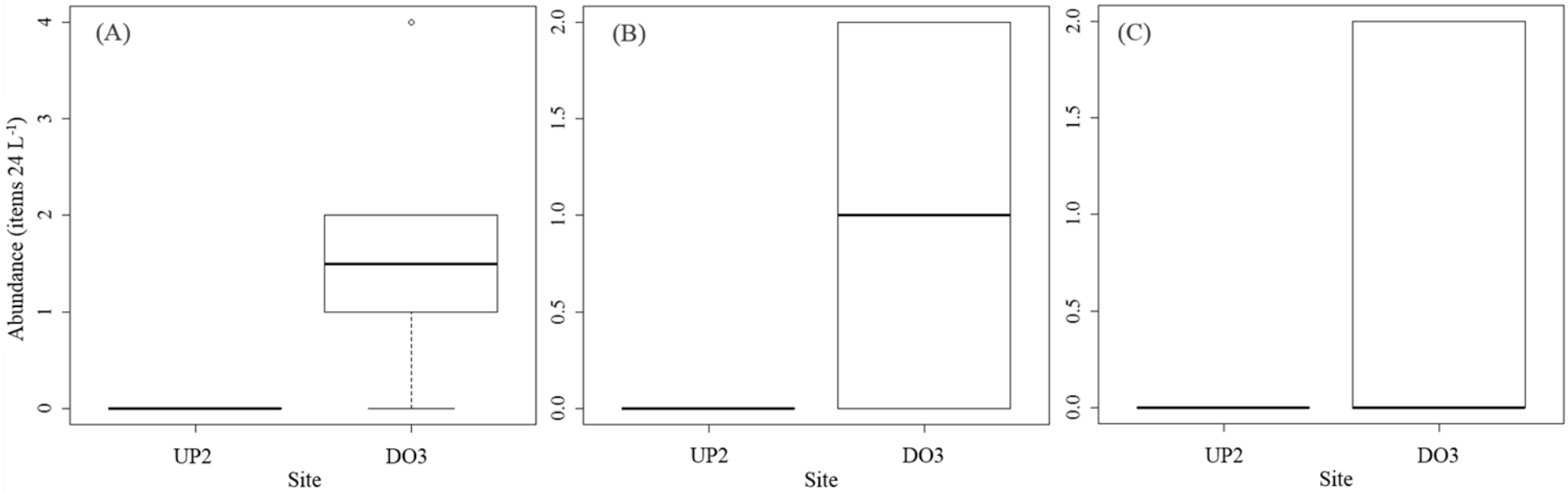


Figure 6-6 Microplastic abundances in spatial sediment samples from the River Clyde for all items (A) and by type: fibres (B), and fragment (C). Pellets and films were not observed in water

6.4.4 Projected sources

Forensic characterisation of MPs was used here to infer the possible contribution from the WWTP and other sources in the catchment.

As most pellets could not be characterised chemically, and they were observed in small numbers in both upstream and downstream sites, it was not possible to infer their specific origin. The presence of microspheres in the environment is attributed to their use in cleansers, cosmetics, and industrial air-blasting, and their subsequent delivery to rivers via WWTPs (Klein et al. 2015; Mani et al. 2015; Leslie et al. 2017). A recent study in the Irwell and Mersey rivers in the UK (Hurley et al. 2018) also observed dominance of spheres in sediment samples, where they noted that their abundance increased in proximity to WWTPs and CSOs but decreased in areas away from these point sources (Hurley et al. 2018). This contrasts with findings from the Clyde, where beads or spheres were consistently minimal at all sites despite presence of similar types of point sources, highlighting the challenges of translating current findings to freshwater systems in general. Given the low recovery rates of standard beads observed in the recovery tests, the possibility that these items were underestimated in this study cannot be excluded.

The higher concentrations of MPs in sediment and water samples at DO3 vs UP2 could indicate WWTP inputs, especially because films and coloured fibres observed in downstream sites were similar to those at various stages in the WWTP. Microfibres are often associated with their release from textiles and Pest and PA are cited as the most common types of MP fibre in environmental samples (Wen et al. 2018). However, here only one coloured fibre could be identified as PA in river water samples, but these were not present in wastewater. Fibres identified as PP and PET/Pest in river sediment could be linked to their transport by WWTPs, based on chemical data alone. However, when combined with visual information, most of the fibres observed in the Clyde did not resemble those released from synthetic garments. Their origin is more likely from recreational fishing in the catchment, which has also been noted as an important source of MP fibres in other river systems (Cole et al. 2011; Zhang et al. 2017). This combined assessment is important because it emphasises that further MP research to establish control strategies cannot rely on just one piece of information. Alternatively, the lab controls suggest that coloured fibres could be associated with airborne deposition from the general environment. Therefore, while coloured fibres were the main MPs present in treated effluent, and WWTPs are often cited as major pathways for fibre release (e.g. Dris et al. 2015; Horton et al. 2016; Di and Wang 2018), here it is not possible to establish if the WWTP is the main contributor of microfibres to the River Clyde.

Microplastic fragments included PP, PA, and PET/Pest, which are common plastics with a wide range of uses including packaging, textiles, and engineering applications. Sheet-like films were classified as vinyl chloride and PBT that may be representative of industrial uses. Therefore, the proximity of residential areas, the industrial park, recreational areas, and major highways could explain the consistent distribution of fragments across all spatial samples but tracing of their specific sources need forensic approaches for assessment and quantification of local activities. A previous UK study observed that transitions from suburban to urban areas were reflected in small-scale

changes of MPs (Hurley et al. 2018). However, detailed assessment of these transition zones was not possible here because all points were located in an urban catchment and are exposed to multiple CSOs and similar land use along the sampling stretch.

Small blue fragments in sediment physically resembled those observed in Daldowie wastewater in stages other than final effluent. These fragments were also categorically similar to those reported in WWTPs in Finland (Talvitie et al. 2017a; Lares et al. 2018) and in California, USA, where they were determined to originate from toothpaste (Carr et al. 2016). Furthermore, several hexagonal-shaped fragments were observed in river sediment, and while these were not found in raw or treated water at Daldowie, similar pieces were reported in sludge cake fractions of a secondary WWTP also discharging to the River Clyde (Murphy et al. 2016), which could propose a link between these particles in the river and WWTPs and highlights the opportunities and need for inter-study collaborations. Furthermore, while final effluent from the WWTP may not be the main contributing source of MP fragments or films to the recipient channel, there may be some associations between MPs in the plant and the river. For example, both the treatment plant and the River Clyde may be exposed to pollution from similar diffuse sources such as degraded plastic debris in urban runoff (Di and Wang 2018). Additionally, the presence of fragments and films in the early treatment stages in the WWTP may also indicate possible discharge of MPs by storm overflow channels. However, these were not sampled during the project and thus their contribution should be explored. Lastly, the resemblance between some pieces observed here and those in sludge in other studies may also happen as WWTPs are also a channel for indirect sources of these materials. Sludge application may result in accumulation of MPs in agricultural soils, and surface runoff from these fields may introduce plastics to catchment drainage systems (Corradini et al. 2019). However, the visual comparison of pieces observed here with those reported in previous studies can only be based on a categorical interpretation of photographs. As fragments can be produced from the breakdown of larger pieces, the predominance of these pieces in rivers is of concern as their source of origin include a wide range of possibilities, making them harder to trace and regulate. Furthermore, pollution is likely to be catchment-specific and therefore it is crucial to generate further datasets to explore spatio-temporal variations of different types of these MPs.

6.4.5 Retention and release of MPs

The higher abundance of MPs in sediment provided evidence of retention of these contaminants by the system. Furthermore, differences across spatial samples may be influenced by changes in sediment composition (Horton et al. 2016). Therefore, to further explore the potential retention and release of MPs in this system, sediment-MP associations were explored by grain size fractionation. The majority of MPs were observed in size classes >0.3 mm, especially between 1.0 and 0.3 mm (**Figure 6-7**). Primary MPs in the form of pellets were most abundant in the 1.0-0.3 mm size class, with only one pellet each in the 0.3-0.18 mm and <0.06 mm fractions. This is consistent with findings in the River Kelvin where pellets were mainly associated with mid-range sediment fractions. A previous study in the Rivers Rhine and Main in Germany similarly observed a predominance of spheres in sizes between 0.63 and 0.063 mm (Klein et al. 2015).

Fibres also concentrated mainly in the 1.0 to 0.3 mm size class, although they were also observed in the 2.8-1.0, 0.18-0.06, and 0.3-0.18 mm fractions, in order of abundance (**Figure 6-7**). These findings differed from those in the River Kelvin, where the <0.06 sediment fraction had the highest concentration of fibres compared to any other size class. The differences between the two rivers may be explained by the nature of the microfibers observed in the two systems, as fibres in the River Kelvin resembled those that would be released from use or washing of clothes, while those in the Clyde are presumed to originate from fishing lines. Fragments were associated mainly with mid-sized sediment between 1.0 and 0.3 mm, while films were present in equal abundance in the 2.8-1.0 and 1.-0-0.3 mm size classes. A few fragments and films were observed in the three smaller sediment fractions, but these were comparably lower than the larger size classes. This provides new information on the possible distribution of fragmented MPs in the catchment as no patterns were identified in the distribution of fragmented MPs in the River Kelvin study, perhaps due to the low numbers of fragments observed in that study.

Separation of MPs into different size fractions is sometimes incorporated into extraction protocols, but the size classifications vary across studies with the majority using two or three size classes, and only few employ four or more (Zhang et al. 2015; Estahbanati and Fahrenfeld 2016; Wang et al. 2017; Di and Wang 2018). Furthermore, the studies that use multiple size classes focus mostly on surface water samples, thus limiting the possibility of exploring sediment-MP associations. Sediment-MP associations represent an area that has not been explored in detail by previous studies, but is important as can provide information on spatiotemporal parameters that can be used to develop and calibrate transport models similar to those available for sediment and low-density particles (Nizzetto et al. 2016; Kooi et al. 2018). Doing so can advance understanding and prediction of their fate and transport and inform control strategies. A modelling exercise was initially planned for this study but due to time constraints and the long processing times, it was not possible. Nevertheless, it is recommended that this is considered in future experimental design in the Clyde catchment and similar freshwater studies.

While MP abundance in surface water was low, presence of MPs indicates that their transport may be continuous even during low to medium flows. Given the highest abundance of MPs in surface water, this could reflect a load of 3×10^8 to 6×10^8 MPs for the flow profile considered here. An attempt was made in this study to explore seasonal variations by incorporating sampling across five months and sampling both sediment and water samples. Concentrations were generally higher in sediment sampled August 2 2018, when the lowest flow was measured for the period of study. However, the next sampling event was only two weeks apart and only two replicates were collected for each campaign. Therefore, temporal patterns of retention and transport could not be assessed in detail here because flows were comparable and did not capture extreme seasonal flows. Seasonality has been discussed as an important driver of changes in MP concentrations (Hurley et al. 2018; Rodrigues et al. 2018). During high flow conditions, higher MP abundances may be expected in surface water due to sediment resuspension and flushing of MPs from these sediments (Hurley et al. 2018). Additionally, more transport of MPs may be expected from the drainage basin to the river channel due to storm overflow systems like CSOs and overflow channels inside the WWTPs, as well as greater surface runoff from the riverbank. More information is needed for conclusive assessment

of the seasonal drivers and the relationship between small- and large-scale variations in hydrodynamic and depositional environments and MP concentrations.

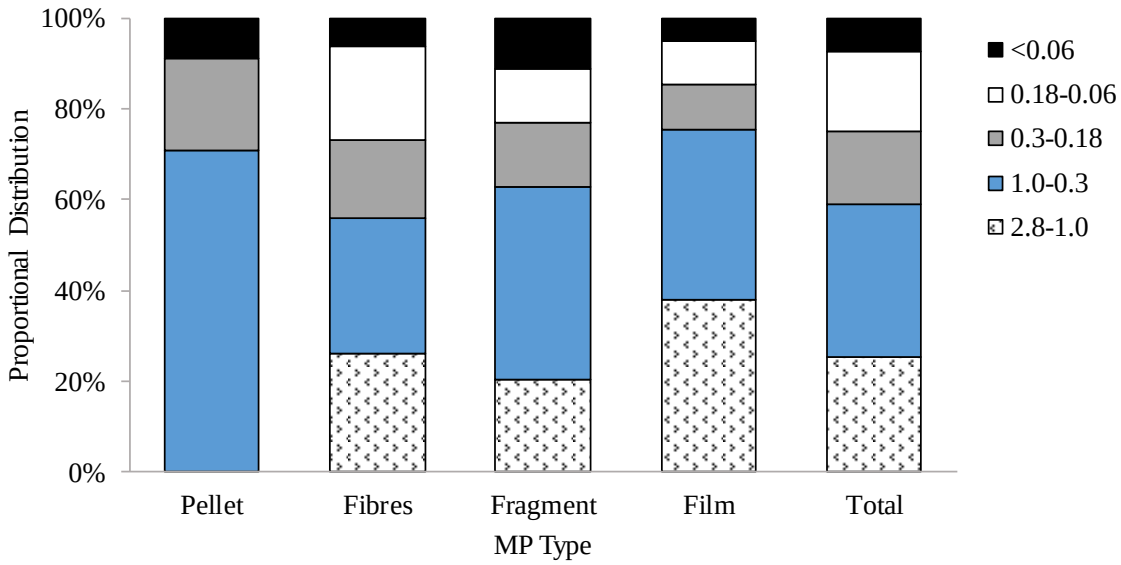


Figure 6-7 Proportional distribution of MPs by sediment grain size class.

6.5 Conclusions

This part of the research focussed on assessing the characteristics and spatiotemporal abundance of MPs in sediment and water samples from the River Clyde, and using this information to explain the role of the selected WWTP and other point and diffuse sources in delivering contaminants to the river channel, and the potential retention and release of MPs. Similar to the two systems discussed in the previous chapters, MPs were continuously present in varying abundances and secondary MPs were predominant. Significant differences in spatial distribution of total MPs and fibres were observed between UP2 and DO3 sites, and overall concentrations were higher downstream. Sediment MP concentrations here indicated comparably lower pollution levels than similar studies elsewhere and provided evidence of retention of MPs. Water MP concentrations could not be compared directly with previous studies, but were considered to be low based on the flow conditions considered in this study and that both small MPs (<63 µm) and large MPs (2.8 µm) may have been missed by the sampling approach. Nevertheless, the presence of MPs in surface water suggests that transport of MPs, especially secondary types may be continuous. However, more data for high flow events in this study site should be collected to explore the role of hydrodynamic and sedimentation processes in the retention and release of different types of MPs.

Fibres were the only MP type observed in WWTP effluent over five sampling events. There were similarities in some physical and chemical traits of fibres between the two systems so a WWTP source to river MP loading cannot be excluded. Similarly, there was some resemblance between films and fragments observed downstream and those observed in the WWTP in earlier stages, but as these

were not observed in final effluent, discharge from storm overflow channels may be the source. That diffuse sources of pollution contribute to the MP loading is further supported by the presence of different types of fragments in similar distribution across all sampling sites, and by the presence of fibres that may be from recreational fishing. This is important for regulatory conversations as diffuse sources may be harder to trace and regulate, especially since pollution is site-specific and the solutions adopted for one location may not be useful in another. This emphasises the need to generate more information for these systems before implementing control strategies that may be costly but ineffective. Overall, the MP concentrations observed in River Clyde sediment were lower than those reported in previous studies in Germany and the UK, suggesting there is less intense pollution in this system. However, further spatial and temporal datasets, higher sampling volumes, and more replicates are still needed for the River Clyde to assess variations associated with changes in watershed characteristics (e.g. population density, land use) and seasonality. Additionally, future research design should consider incorporation of spatial samples of CSOs, storm overflow channels from the WWTP, and tributaries. Addressing these remaining gaps can provide a more comprehensive evaluation of this river in order to identify sources and mitigate MP discharge.

Overall, it has become well-established in recent years that MPs are abundant in aquatic environments and remote areas worldwide, so it may no longer be surprising to find presence of MPs in nearly every environmental compartment sampled. However, environmental concentrations are highly variable and thus MP quantification and characterisation studies remain crucial as it is difficult to measure risk unless the full extent of contamination can be quantified, especially for the small fractions that may be more abundant and more easily ingested. This variability may be difficult to quantify on a global scale at present, but research could begin to focus on developing comprehensive descriptions of variations in local catchments by expanding their spatiotemporal scales. Therefore, this research can contribute to risk assessment of MPs in freshwater systems as it generates extended spatiotemporal information on their concentration. However, MP concentration is only one component of risk assessment and thus needs to be complemented by further ecotoxicity information.

Risk assessment for other contaminants traditionally employs measures of the magnitude of concentration, exposure due to contact, and toxicity effect (Lambert and Wagner 2018). However, for MPs, risk assessment is challenging because the extent of pollution has not been fully quantified and because, even though MPs are considered harmful contaminants, the ecological risks of MPs are largely unknown. Negative effects have been observed at the individual and population levels, while several studies have reported that MPs are efficiently egested and that no or mixed results are observed from exposure (Anbunami and Kakkar 2018). A recent modelling study based on estimates of marine MP concentrations, predicted that negative effects of MPs may not happen until 2100 at concentrations exceeding 6650 particles m^{-3} , but this did not consider the toxicity effects of added contaminants (Everaert et al. 2018).

However, as MP generation grows, the order of magnitude of MPs is likely to increase, potentially resulting in elevated exposure, particularly for secondary MPs. Research considering dosage effects observed higher concentrations were associated with greater uptake and some detrimental effects, but often these returned to normal upon reaching a threshold (Anbunami and Kakkar 2018). These

dosage studies may suggest that similar to other emerging contaminants, an increase in MP concentrations may have a negative impact, but this comparison may be limited because unlike other emerging pollutants, the effect of MP exposure may depend not only on mass but on physical and chemical properties of the material (Lambert and Wagner 2018). Additionally, often exposure studies are lab-based and use concentrations of MPs higher than those in the environment and in absence of other food sources, so adequate quantification of environmental concentrations is necessary to understand how lab bench findings translate to field conditions.

To improve risk assessment of MPs, dose-dependent effects should be investigated for different categories of MPs as scientific evidence is not yet sufficient to determine the most important types, sizes, and shapes of MPs that pose a risk to humans and other organisms. Furthermore, characterisation of MPs by shape, size, and colour is important because these may help to predict their uptake by organisms and their toxicity. For example, certain sizes and colours could be confused as food by organisms, while certain colours may be associated with specific additives or co-contaminants (Anbunami and Kakkar 2018). Further, it has been suggested that small MPs and nanoplastics may behave similar to engineered nanoparticles, being able to pass through cellular membranes and carry other toxic chemicals and metal ions (Syberg et al. 2015). Nevertheless, risk assessment approaches for nanoparticles and other emerging contaminants assume homogeneous distribution of the contaminants in the environment (Lambert and Wagner 2018), which is not the case for MPs and thus current risk assessment approaches may not be directly applicable to MPs as a single category. Therefore, there is currently a need for further ecotoxicity studies to identify the specific metrics needed for risk assessment of different types of primary and secondary MPs, especially for freshwater rivers that are complex and dynamic systems for which limited knowledge is available.

7 Summary and Conclusions

The issue of plastic pollution is not new, but the cumulative generation of plastic litter since the onset of mass plastic production has increased the scale of the problem to a global crisis. Furthermore, new information on different types of plastic litter has raised increasing concern over smaller plastic fractions or MPs and prompted considerable research on the topic of MPs pollution of aquatic systems since 2004. Similar to other anthropogenic waste products, the majority of plastic litter originates on land (Rochman et al. 2015) with oceans as the final destination, thus research efforts initiated in and continue to focus on marine systems, leaving in-land water bodies largely unexplored. Wastewater treatment systems and rivers are known transport vectors of anthropogenic contaminants, including MPs, thus warrant further attention as their study can provide understanding of how to regulate discharge of these materials to the environment. Hence, this research aimed to explore MPs occurrence, distribution, and fate in wastewater and freshwater rivers to generate insight on the role of these systems as linkages between MPs sources of origin and marine debris, thus helping inform policy.

When this research was started, worldwide studies that considered MPs distribution in inland water systems and their connection with WWTP discharge were limited and there were no published fresh- or wastewater studies for the UK. The first publication of MP pollution in such systems in the UK was from a wastewater study in Glasgow in 2016 (Murphy et al.), with studies for English freshwaters following in 2016 (Horton et al.) and 2018 (Hurley et al.). Over the past four years, research on freshwater rivers and wastewater systems has grown internationally, but the field still lacks empirical data that can help build a comprehensive understanding of the extent and distribution of MP pollution. While MP studies may run the risk of appearing as just one more data point in a series of disconnected studies, due to the high variability of MPs in the environment and the still scarce number of publications for freshwater rivers and wastewaters, further generation of local datasets is necessary to fully understand the extent and variability of MP pollution. Furthermore, it is well established now that MPs are ubiquitous so the research presented in this thesis supports using in-depth examination of local datasets to understand small-scale variations from extended datasets and infer local points of entry, which is essential for reducing MP pollution from its source. Moreover, adequate quantification of the abundance of an environmental contaminant is an essential component of risk assessment, along with exposure and toxicity data.

At the start of the PhD, the following questions were identified relating to fresh- and wastewaters: (1) What is the level of MP pollution in a WWTP and its recipient waters?; (2) What is the efficacy of the WWTP in removing MPs?; and, (3) Can sources and transport of MPs in rivers be traced? To explore these, this research focussed on the River Clyde catchment in Glasgow, Scotland, with the overall aim of evaluating the quantities, types, and distribution of MPs in three interconnected water bodies, as well as the fate of MPs as they move through various compartments in this River Clyde system.

This final chapter consolidates the main findings from the three empirical chapters according to the three specific objectives of this PhD research stated in Chapter 1. Final consideration is given to

methodological challenges, the main research contributions, and future activity required to resolve some of the remaining research gaps.

7.1 Objective 1 conclusions: What is the level of MP pollution in a WWTP and its recipient waters?

The first objective of this research was to quantity the main types of MPs present in a WWTP and freshwater rivers in an urban catchment located in close proximity to the ocean, in order to assess the level of MP pollution in these systems. This objective was addressed by studying three interconnected systems in the following order: a freshwater urban river (River Kelvin), a large tertiary WWTP (Daldowie), and a large freshwater river receiving discharges from the previous two systems (River Clyde).

7.1.1 MP concentrations

Microplastic pollution was prevalent in all systems, indicating a continuous input of these pollutants to the environment, which is consistent with general notions from the MP literature of their widespread distribution across aquatic systems worldwide. Also consistent with previous findings is that MPs displayed high intra- and inter-site variability at a small and large scale. A total of 200 L of wastewater were processed across eight treatment stages in the WWTP and five sampling campaigns. Wastewater contained between <1 and 13 MPs L^{-1} in raw and treated water across different stages of the WWTP. The higher MP concentrations were observed in raw (10.1 MPs L^{-1}) and pre-treated (12.4 MPs L^{-1}) wastewater. Treated effluent contained between 0.1 and 0.4 MPs L^{-1} in the morning samples, but the afternoon sample had 2.3 MPs L^{-1}, indicating there may be short-term fluctuations that should be explored further. Concentrations observed at Daldowie are comparable to MP concentrations of similar size measured in a nearby Scottish secondary WWTP in the same catchment, where influent was observed to contain ~15 MPs L^{-1}, while treated effluent contained 0.25 MPs L^{-1}. Influent and effluent concentrations here were also comparable to those in a tertiary treatment plant in Italy (Magni et al. 2019). Influent concentrations are on the lower end of those reported elsewhere, while effluent concentrations are similar to those in other tertiary sites, noting that direct comparison may be limited due to differences in methodology. For example, 388-686 MPs L^{-1} and 0.7-3.5 MPs L^{-1} were observed, respectively, in influent and effluent of a Finnish WWTP considering a smaller size cut off size of 20 µm and sampling with an electric pump and on-site filter set up (Talvitie et al. 2017). The higher abundance observed in the Finnish study and lower cut off may suggest that the lower limit of 60 µm at Daldowie may lead to an underestimation of MPs by missing the smaller fractions.

Storm overflow channels from the main WWTP were not sampled here but as MPs were observed in higher concentrations in the early treatment stages, discharge from such channels could also serve as a pathway for delivery to the river during high flow events, and thus warrant further exploration. Sludge fractions were not sampled in the main WWTP but the activated sludge samples from Shieldhall, a nearby secondary WWTP in the same catchment as Daldowie, also contained MPs. This supports previous inferences that MPs are removed in biosolid fractions in wastewater treatment

facilities, although most observations come from primary treatment solids with limited information on other sludge fractions.

The river environments also contained MPs in sediment and surface water. Concentrations in the River Kelvin tributary were 161 and 432 items kg^{-1} across two sediment sampling events collected two months apart from the same spatial point. These MP concentrations were higher than in sediments of the Clyde, which contained between 1 and 26 items kg^{-1} across duplicates, obtained from five spatial samples and three separate campaigns 3 weeks to 2.5 months apart. Larger rivers are expected to have higher pollution (Galgani et al. 2000), but these differences in sediment MP abundances suggest that the smaller river was more polluted. Since the Kelvin joins the Clyde estuary approximately 30 km from the discharge of the Clyde into the Atlantic on the west coast (at Greenock), MP pollution in the River Kelvin could contribute to the MP loading to the marine system if not remediated. The differences between the two rivers examined here may be a result of the high spatio-temporal variability of MPs in the environment and indicate that the extent of MP pollution is localised and thus datasets should be generated for discrete catchments.

Nevertheless, the differences between the two rivers may also be a result of the different methods used for chemical characterisation for each study. The Kelvin study was conducted in 2015-2016 and FTIR techniques at the time were relatively new, thus the use of visual sorting followed by SEM-EDS to discriminate against inorganic materials, reflected methodological trends. However, visual and SEM-based techniques assumed that more than 90% of items were MPs since electron microscopy did not provide the adequate tools for confirmation of plastics. When the Clyde study was conducted in 2018-2019, FTIR analysis was the main technique used for chemical analysis. Based on FTIR results, it was determined that cellulose was the second most abundant material identified in visual counts and therefore easily mistaken for MPs. Cellulose would not be separated from plastics by SEM-EDS characterisation since both are C-based materials. This may be part of the reason why MP concentrations were estimated higher in the smaller tributary. For example, in this research, 39% of subsampled specimens from River Clyde sediment were identified as synthetic polymers. If this correction factor was applied to the Kelvin counts, MP concentrations would be lower: 63 to 169 MPs kg^{-1}. Nevertheless, concentrations in the Kelvin are higher than in the Clyde even after this correction is applied, thus still indicating a higher MP pollution level in the smaller river. Further replicates and sampling across different season for both sites are required to confirm the magnitude and patterns of MP pollution throughout the two rivers. The FTIR-correction employed here highlights that as MP research advances, new techniques are needed as part of routine testing to produce more accurate MP estimates and avoid overestimation of pollution levels. Where not possible to analyse all suspected MPs chemically, the use of chemical data from a subsample for data correction should be considered.

Concentrations in the River Clyde sediment were lower than those reported in freshwater rivers elsewhere (e.g. Klein et al. 2015; Hurley et al. 2018; Rodrigues et al. 2018), but only three studies are available for direct comparison considering the methods and size ranges. Moreover, maximum concentration in surface water was 4 MPs in 24 L of water sampled, roughly equal to 166 MP m^{-3}. These concentrations were higher than others (e.g. Dris et al. 2015; Kataoka et al. 2019) but noting

that these differences may be artificial as most studies use net sampling and consider different sizes and volumes. However, concentrations in River Clyde water were lower than those in Chinese rivers that sampled similar volumes using a Teflon pump and sieve setup with a comparable cut off size of 48-50 µm (Wang et al. 2016; Di and Wang 2018). The presence of MPs in Clyde water indicates that these materials can be transported even under low to medium flows sampled in this research.

The results of this research supported the hypothesis: *MPs will be present in urban freshwater rivers and a WWTP in different shapes, sizes and polymer composition*. Therefore, the first hypotheses for each study were also supported as MPs were present in all sediment, water, and incoming wastewater samples. Further research is needed to incorporate information on sizes that were not measured by the research protocol (e.g. < 60 µm, and >2.8 mm) to provide a more comprehensive estimate of MP pollution. Furthermore, as most of the samples were capture during low to medium flows, high precipitation events need to be sampled in this catchment to assess how MP concentration and distribution may be affected by seasonal changes. This may provide more insight on MP fluxes across these systems.

7.1.2 MP types

Morphology and composition of MPs were assessed by visual sorting and chemical analysis. The stepwise characterisation approach and use of both SEM and FTIR techniques supported the second hypothesis in Chapter 4 that *chemical analysis will improve MP characterisation*, showing that different techniques have both advantages and limitations for different types of MPs. However, this hypothesis would benefit from additional information for other techniques not employed here but used elsewhere (e.g. Raman, GC-MS).

Microplastics were present in different primary and secondary types, sizes, and polymer composition. Fibres were dominant in all three water bodies, which is consistent with general findings from the literature that report high fibre concentrations in WWTPs and in rivers that receive wastewater effluent discharges (Magnusson and Noren 2014). However, the polymer composition of fibres in the River Clyde and Daldowie was variable and uncommon. Visual and chemical characteristics of fibres observed in River Clyde sediment were used to infer that most of these particles likely originated from recreational fishing, with few possibly associated with WWTP discharge. Polyester and PA fibres were not observed in the treatment plant and only one nylon fibre was observed across all river sediment and water samples. The lack of Pest and PA may be due to methodology rather than their absence from the systems as these materials are the most common types of fabrics used for garments and are released in high quantities in washing machine effluent (De Falco 2018). The predominance of PP as the most detected fibre material may be because these particles had higher length and diameter than pieces that were easily lost, thus they were easier to manipulate for chemical identification. However, the presence of PP fibres in wastewater may be explained by their use in hygiene products, medical fabrics, cigarette filters, non-woven thermal clothing, and construction materials (Mandal 2019). Therefore, the abundance of PP fibres in the WWTP is an important finding because it highlights the role of alternative sources of fibres to these systems that so far are seldom discussed in the literature.

These findings only partially support the second part of hypothesis 1 for Chapter 5 that MPs in incoming wastewater would be *especially microbeads released from personal care products and fibres from washing machine effluent*. While MP bead concentrations were almost negligible, fibres were indeed the main contributors to incoming MPs as predicted, but these were not composed of Pest/PA which are cited as the more common types of fibres released from washing of synthetic clothes. The general absence of microbeads was explained as a possible outcome of recent actions to phase out their use in personal care products. Here, a characterisation of microbeads in personal care and cleaning products sold in local supermarkets, and market data on their use in household may help to find the more likely explanation for this. Since Pest and PA fibres are observed in every other wastewater study, their limited presence in Daldowie lead to the interpretation that this may be artificial due to the sampling and identification protocols, as described earlier. If indeed PE/PP microbeads and Pest/PA fibres are in low concentrations in this particular system, then the 5 L sampling volume and size limits of FTIR-ATR confirmation may have resulted in their underestimation.. It would be recommended to repeat sampling at the WWTP using higher volumes and complementary sampling and characterisation approaches to determine if these findings are due to local catchment variations or due to the methodological approach.

Fragments were abundant, especially in the Clyde but it was not possible to infer their source of origin since they were mostly composed of PP and PE and these materials are employed in manufacturing most items of daily use. Copolymers of PE including PE-PP and EVA were also observed in the WWTP and the River Clyde. These copolymers can have commercial value as they can be used in a variety of applications (e.g. EVA is used as an adhesive or sealant), and thus their presence may indicate industrial origins (Ronca 2017; Wang et al. 2017b). From a policy standpoint, copolymers may be important for regulators as they open the door to engineering new blends of synthetic plastics to suit specific demand, and thus have the potential to increase plastic production.

Overall, the dominance of secondary MPs is consistent with most studies and emphasises that these materials are more abundant in the environment than primary MPs. The low abundance of primary MPs in the three sites observed here is important because earlier research stressed the abundance of microbeads in oceans and the need for source reduction of microbeads (Rochman et al. 2015). Therefore, the finding from this research, that primary microplastics are not prevalent may be as voluntary and regulatory bans (in Europe and the UK) have caused a reduction in such materials. If so, outlook is positive for the potential to control discharge of primary MPs to the environment, and other MPs if their specific sources can be identified. Therefore, more emphasis should be placed on the use of forensic approaches for improved characterisation of secondary MPs to pinpoint their source of origin in individual catchments and target mitigation strategies.

7.2 Objective 2 conclusions: What is the efficacy of the WWTP in removing MPs?

Despite recent advancements in knowledge, the role of WWTPs in removing MPs remains inconclusive because removal efficiencies are variable and studies are limited. Therefore, the second objective of this PhD research was to estimate loading to and from the WWTP and assess the efficacy of the sewage treatment process to retain MPs. Incoming MP concentrations were 2.5 to 10.1 MP L^{-}

[1], with an average of 5.8 MP L^{-1} equivalent to 8.1 x 10^8 incoming MPs day^{-1}. In comparison, a secondary Scottish WWTP located in the same catchment as Daldowie, received an average incoming load of 4.1 x 10^9 MPs day^{-1}. The tertiary treatment at Daldowie consisted of trickling filter technology using plastic sheets, thus reporting on a new technology that has not been previously considered. This tertiary treatment was found to be highly-efficient in processing particles larger than 60 µm, despite using plastic media. Despite a retention efficiency of 96% of incoming particles, MPs may still be contained in treated water and represent a daily discharge of millions of MPs to the recipient channel. For example, an average effluent concentrations of 0.2 MP L^{-1} at Daldowie equalled to 2.2 x 10^7 MPs day^{-1}. This daily discharge of millions of MPs in treated effluent is consistent with previous reports (Sun et al. 2019).

Given the size of the population served at Daldowie, incoming and outgoing loadings, respectively, are roughly equal to per capita loads of 4.4 x 10^3 MPs $inhabitant^{-1}$ day^{-1} and 1.2 x 10^2 MPs $inhabitant^{-1}$ day^{-1}. While per capita loads were not reported in the previous Scottish study at a nearby secondary WWTP, based on the information they provide, these are projected to equivalent to 6.3 x 10^3 $inhabitant^{-1}$ day^{-1} and 1 x 10^2 $inhabitant^{-1}$ day^{-1} for influent and final effluent, respectively. Therefore, while daily incoming loading and population served were lower at Daldowie than the nearby WWTP, when normalised per capita, the contribution to and from both sites are in the same order of magnitude, despite Daldowie having an additional polishing step. Following this assessment to report percentage removal and normalise per capita loadings may improve comparability across sites.

The comparison of incoming vs. outgoing MPs (adopted here) is the only way to determine the efficacy of current treatment processes to remediate incoming MP pollution. Thus, this research is novel as very few studies so far sampled treatment stages other than final effluent. This research is consistent with other observations that most MPs are removed by the processing of wastewater. For regulators, this raises the further questions: (1) What is the minimum level of MPs in treated effluent?, and (2) How to bridge the gap from 96% to 100% removal, if necessary? The first question can only be addressed by further ecotoxicity research. However, for deeper insight into the second question, stepwise sampling can help establish how to take advantage of WWTPs in regulating MPs discharge to the receiving waters, by identifying which technologies display higher performance in removing MPs.

Here, samples were collected at each step of the process to investigate the effectiveness of each treatment stage to retain MPs. Processing wastewater from influent and early treatment stages is difficult and often large volumes cannot be sampled, as found here. Nevertheless, a spatial sampling approach can help researchers and regulators understand if treatment technologies already exist to deal with MP pollution, how current processes can be adapted to make them more suitable to remove particles of different types and sizes, and if new solutions need to be engineered. As this study was in collaboration with SEPA and Scottish Water, the research design for this study was to produce information to aid in identifying control strategies. For example, as most removal was observed by the primary stage and previous studies report MPs in primary sludge (Bayo et al. 2018; Li et al. 2018), future research efforts may focus on quantifying the proportion of MPs retained in sludge against the proportion of MPs that may fragment into smaller particles and may be redistributed in the system.

As observed here, fragmentation of larger MPs beads (>700 µm) in controlled tests was observed within minutes of processing of wastewater samples. Similarly, a recent study in lab controlled tests showed that generation of nanoplastics occurred within five minutes of mechanical treatment of PS (Ekvall et al. 2018). However, understanding of fragmentation rates of different plastics under various conditions is limited and thus this fragmentation hypothesis should be tested further. Moreover, for MPs contained in sludge, it is necessary to focus research efforts in understanding the role of sludge treatment plants as control points to avoid release of MPs from mismanagement of biosolids. Secondary treatment removed more MPs, but systematically, during a one-day sampling, P4a and P4b concentrations were different, and P4 samples also differed from P5 (**Figure 5-5**). There is no additional treatment among these three points, so this may suggest that additional settling or breakdown of particles may take place as the water flows through the two streams and to the redistribution chamber before final treatment. This is an important finding because the separation of the stream into two equal channels, called here "phases", and the subsequent mixing of the liquor from two phases is unique to this site and suggest that other factors (e.g. site-specific engineering parameters and infrastructure) besides MP loads and type of treatment play a role in MP retention.

The use of a tertiary polishing step at this WWTP appeared to remove an additional portion of MPs not retained by secondary treatment. However, employing a tertiary treatment may not be feasible in every site and the information on advanced treatment efficacy is still too limited to determine to what extent advanced technologies may be useful. Even though tertiary WWTP research is limited and contrasting findings have been reported, there seems to be a general pattern in predicting removal depending on the pore size of the filters. For example, membrane bioreactor technologies based on ultrafiltration may be expected to display better performance than plastic filters and than slow sand filtration. However, plastic equipment efficiency may decrease with time and the plastic media may also degrade, thus the volume of MP generation could increase due to lack of retention and by direct generation. The likelihood of this happening is unknown, but needs to be considered and so is a research need. Ongoing temporal monitoring of effluent is time-consuming, but water quality is routinely monitored and it would be interesting to explore if there is a link between changes here that indicate this final scrubbing is losing effectiveness and MP generation. The generation of MPs from the plastic media can be monitored by physical and chemical characterisation of the filters at the tertiary treatment stage to determine if similar or different than MPs observed in effluent.

Primary and fragmented MPs for the sizes considered here were almost entirely removed from the system, but individual fibres were difficult to capture and were the main type of MPs to persist until final effluent. The presence of fibres in final effluent may be due to their ability to pass through filters longitudinally (Sun et al. 2019). However, during extraction and characterisation, the fibres were observed to form clusters. Thus future research could explore treatment technologies that promote their aggregation into bigger composites and make them easier to remove from the system. Similarly, new research may consider ways to promote aggregation of other fragmented pieces with biofilms for sinkage of materials that make MPs easier to remove from the system. The information that is currently available on MPs for the water sector may not be sufficient yet to pinpoint the specific technologies needed to regulate MPs and thus this research can be used as a basis to guide further spatio-temporal research. It would be good if the sampling at Daldowie can be replicated for higher

volumes and number of samples, and for MPs <63 µm and >2.8 mm, to confirm the observations here are temporally stable before control points are established.

Based on these findings, the general hypothesis 2 and hypothesis 3 of Chapter 5, respectively, were also accepted: *Wastewater treatment will remove a portion of MPs, but not all*; and, *MPs concentration will decrease after each treatment stage, but some discharge may still occur*. However, more replicates per sampling point are needed to determine if the reduction after each additional treatment stage is statistically significant and how this may fluctuate on a daily basis. Also, sampling of sludge and other biosolid fractions produced after each step would be required for a mass balance analysis and to determine if these particles are being removed in sludge or can be cycled back into the system.

7.3 Objective 3 conclusions: Can sources and transport of MPs in rivers be traced?

The final objective of this PhD project was to assess the potential fate of MPs in the recipient freshwater river channel and explore the possible associations between MPs and point and diffuse sources of pollution. The River Clyde was contaminated with MPs, likely originating from point and non-point pollution for, as well as receiving discharge from the WWTP and CSOs, it also drains a large catchment, with urban and rural land use, and receives water from various tributaries. The profile of MPs in the River Clyde had differences and similarities with effluent water. For example, the majority of fibres in the River Clyde were clear PP fibres resembling those used as fishing lines, whereas most of the fibres in the WWTP were coloured PP fibres. However, some coloured PP fibres and films similar to those observed in the WWTP were observed downstream from the effluent pipe. Fibres in the recipient channel were primarily observed in sediment, indicating that during low to medium flows, the river may be retaining fibres and therefore, their transport is less likely to occur. Retention of fibres in sediments may be due to their affinity for heteroaggregation with other particles like sediment grains and organic material that promote their settling, and thus their affinity for aggregation in different matrices should be explored further.

Fragments were abundant in the River Clyde compared to the other systems, and were observed in both sediment and water samples. The abundance of fragments in liquid and solid compartments across spatial points indicates that diffuse sources are likely to play a larger role in delivery of these types of MPs. Furthermore, this indicates that the system is actively capturing and transporting fragments of different types and sizes during low to medium flow conditions. Moreover, even though drifting MPs in surface water were smaller compared to those in solid fractions, the River Clyde is a large river that moves large volumes of water and so considerable transport can still occur. For example, at the maximum MP concentration of 4 MPs 24 L^{-1} and based on SEPA's flow data, these concentrations could represent a daily transport of 3×10^8 to 6×10^8 MPs under low to medium flows respectively. The most downstream point (DO3) sampled here was approximately 6 km from the tidal weir, after which the Clyde becomes estuarine, thus further spatial points should be sampled along the estuary to assess retention and release of MPs from the freshwater portion of the river to the ocean. This assessment is important for understanding the inheritance of marine MPs from in-land waters. At the same time sampling should explore seasonal fluctuations in the transport of MPs,

especially during high rainfall events when flushing of MPs and thus higher concentrations may be expected (Hurley et al. 2018). The sequestration of MPs in sediment may not be permanent as sediment will become resuspended during high flows and the likelihood for this to happen could be explored to assess expected export responses to downstream systems after source loading ceases.

While point sources like WWTP may be comparably easier to regulate, rivers are complex systems because they collect materials from the terrestrial environments they drain and this means they can receive contamination from almost any activity or land use. Therefore, controlling river transport will be more difficult and the hydrological and geomorphological factors driving the spatio-temporal distribution of MPs need to be better understood, as managing MP pollution requires comprehensive knowledge of their distribution and fate in aquatic systems. Here, MP-sediment interaction were assessed to explore how the association of MPs with different grain size fractions may influence their settling or transport. However, the research was inconclusive as differences in patterns were observed between the two rivers. In the River Clyde, most particles concentrated around the 1.0 mm particle size regardless of type, while in the Kelvin, fibres were mostly observed in fine sediment ≤63 μm. While the sediment-MP dynamics require further assessment, these observations can be used as a basis for further work especially since size fractionation data can provide insight on MP partitioning and aid in modelling the dispersal and distribution of MPs in rivers.

The third general hypothesis was partially supported: *Spatial distribution of MPs in rivers will reflect the influence of point and diffuse sources of pollution and changes in hydrodynamics*. For example the *increase in total MPs concentrations downstream from the WWTP* was observed, supporting hypothesis 2 of Chapter 6 and leading to the interpretation that increasing concentrations may reflect the effect of WWTP discharge of treated effluent and stormwater overflow. However, the proximity of sampling points and events, limited understanding of how different localised sources of pollution and changes in hydrodynamics may reflect in spatial distribution of different types of MPs, and thus further spatiotemporal sampling in the River Clyde is needed to support this discussion.

Furthermore, the role of the River Clyde as a sink or source of MP was tested via the last hypothesis for Chapter 6: *retention of MPs by river will be reflected in increase in sediment MP concentration and decrease in water MP concentration, with decreased flows*; and, conversely, *transport of MPs by river will be reflected in increase in water MP concentration and decrease in sediment MP concentration, with increased flows*. The sampling scheme was designed to collect data from low to high rainfall events, but unfortunately only low to medium-low flows were captured during the sampling period and therefore the findings did not provide sufficient evidence to fully test these hypotheses. Microplastics were always observed in river sediment, which may provide evidence of retention of MPs during period of low flow. However, these concentrations could not be compared to those during high rainfall conditions. Furthermore, transport of MPs was evidenced by presence of MP fragments and fibres suggesting that transport may be continuous and not limited only to precipitation events. Therefore, the information needed to support these hypotheses is incomplete and sampling for the Clyde should be repeated to capture the full extent of seasonal flows to produce the relevant information to test these further.

7.4 Challenges and limitations

Overall, challenges and limitations for this research were attributed primarily to methodology and, for ease of discussion, are grouped into three broad categories as follows: (1) visual and manual dependency, (2) sampling volume, (3) number of replicates. These have been clearly characterised in the empirical chapters to provide the reader with enough information to know what these mean in terms of variability and accuracy of estimates. However, these challenges are reiterated here as they are common across studies and thus emphasise that more information is needed when reporting protocols to inform interpretation and improve transparency, inter-study comparisons and reproducibility of experimental design.

The dependency on visual inspection of samples as the first step of MP characterisation limited the detection size to what is observable under the microscope and what could be manually collected for FTIR analysis. Therefore, analysis of particles > 60 µm, based on the size fractionation data was only possible and particles smaller than 60 µm were not quantified. Chemical analysis by FTIR-ATR was challenging for particles <300 µm because smaller pieces were easily lost during transfer. These size limits reflect an important limitation as it underestimates smaller MPs that are projected to be present in higher abundance than the larger MPs and therefore may underrate MP pollution in the catchment. The decision here was made to extrapolate the FTIR correction to the fractions 63-300 µm, and while this was considered an acceptable approach to provide more conservative estimates, this calculation is not commonly employed by others and was not validated here for the smaller fractions. Therefore, controlled tests using MPs <60 µm should be conducted to validate the use of this FTIR correction and modify accordingly.

Recovery rates of microbeads and fibre-like standards observed in the validation tests showed that current manual extraction processes may not extract all particles in the samples. Therefore, there is a possibility that certain types of items are underestimated depending on the protocol. While these validation tests were conducted here, it was decided to not correct the sample data for these recovery percentages because standards used for these validation tests, while selected due to their common use in daily products (e.g. microbeads, brush bristles, packaging mesh), were not commonly observed in the river or WWTP samples. Further tests were not conducted here due to time constraints and therefore this is a limitation of the research design that require further particle recovery tests using more representative standards.

Another challenge with visual sorting is that morphology is not always straightforward, especially as particles decrease in size. Here, four general categories were used for sorting but as MPs are highly diverse, more classes may be needed for adequate assessment of the extent of MP variability and relative contributions of primary vs secondary types. For example, the fibres category included what other studies classify separately as lines, while pellets included anything resembling personal care products typically <100 micron but also any larger spheres and oval-shaped pieces. Fragments were perhaps the more difficult category to classify, as other studies tend to use sub-categories such as foams, films, and sheets (Hidalgo-Ruz et al. 2012). The challenge with visual sorting lies in that particles are sometimes difficult to place in a distinct class. This is likely a limitation that all

researchers may be facing but is seldom discussed in papers, and therefore, until automated methods are developed, highlights an urgent need for unified guidelines for visual identification of MPs.

The decision made here to process the entire sample to minimise particle loss meant that sampling volume and number of replicates were low for liquid fractions in both the WWTP and the main river due to extended processing times. A general assumption is that higher volumes are needed for statistically representative results, especially for WWTP effluent as certain types and sizes of MPs may be present in low concentrations and thus sampling smaller volumes could lead to underestimation (Sun et al. 2019). However, the empirical information is not sufficient to establish what these volumes should be (e.g. hundreds or thousands of L). Therefore, until standardised guidelines become available, it would be advised to replicate components of this study for different sampling volumes and more replicates to evaluate MP variability by different method approaches and help guide further research. Furthermore, the long processing times also limited the number of sampling events that could be incorporated for each site. For example, the initial sampling scheme for the WWTP included three replicates per sampling point and monthly sampling over a 12-month period. However, after the first sampling campaign, the sampling scheme had to be modified as it became clear that this would not be possible.

7.5 Contributions to wider research

Microplastics have been found everywhere, from remote to densely-populated areas, and from drinking to wastewater treatment facilities. It is recognised that primary and secondary MP litter is an emerging threat to water resources worldwide and adds pressure on global water supplies. Microplastics will travel from their source to rivers via diffuse and point sources like WWTPs as observed here, and from source to oceans via rivers. Therefore, this research contributed to understanding the role of freshwater rivers and WWTPs as transport vectors or filters of MPs by measuring MP abundances and exploring the interconnectivity between the WWTP and its recipient channel. It has been established that most MPs were retained in the WWTP, but MP pollution in rivers is more complex and has not been fully conceptualised. As WWTPs are enclosed systems, it is possible to control what happens inside these systems through engineering processes, but managing river pollution is difficult and requires more attention. Therefore, the focus of this research on WWTP and rivers is valuable because MP research in these systems is still in its early stages and for which available studies remain scarce.

Moreover, clean-up technologies for MP pollution in the environment are not available, thus the only realistic way to address this issue at the moment is preventing further introduction of MPs to the aquatic environment. Reducing or eliminating the use of plastic from everyday lives may take years (and may not be fully desirable), and even if plastic production and use was stopped completely, secondary MPs would continue to be produced from breakdown of plastic litter already in the environment. While the ecological impacts of MPs remain poorly understood, there is scientific evidence of adverse effects on biota, for example due to disruption of feeding behaviour that leads to starvation and toxicity from leaching co-contaminants (Anbunami and Kakkar 2018). Therefore, there is an urgent need to control land-based sources of these materials in order to start reducing

pollution now, but reduction cannot be achieved unless more datasets become available to establish the quantity and types of MPs present in different compartments. By focussing on extended spatio-temporal datasets from each of the main systems at Daldowie and the River Clyde, this research contributes scientific knowledge on sources and types of pollution, the possible associations with land and water use, and the interconnectivity of three water bodies in the same catchment. This type of information is important for advancing understanding of the extent and variability of MP pollution in the local Clyde catchment. This information can be used by water authorities to infer local points of entry, and inform integrated management strategies to regulate MP discharges to the environment management of individual catchments, but to do this extended spatiotemporal datasets should be generated for interconnected water supplies in individual catchments.

Also, by generating information on MP abundance, this research can contribute to risk assessment of MPs in freshwater systems. Risk can be defined as the likelihood that exposure to a contaminant will have a harmful effect on humans or ecological systems, and requires information on the presence/abundance of the contaminant, exposure, and the intrinsic toxicity of the contaminant (USEPA 2019). Currently, although MPs are considered harmful contaminants, risk assessment of MPs is limited. In terms of MP abundance, it is well-established that MPs are present in aquatic environments and remote areas worldwide, so it is no longer surprising when a study reports their presence. However, environmental concentrations are highly variable and thus so it is difficult to measure risk unless the full extent of contamination can be quantified, especially for the small fractions. This variability may be difficult to quantify on a global scale at present, but research could begin to focus on developing comprehensive descriptions of variations in local catchments by expanding their spatiotemporal scales. Nevertheless, this research did not generate understanding of exposure or ecotoxicity as these were outside of the scope of the study, and thus the present research can be used as one piece of the puzzle for risk assessment in the Clyde catchment.

While development of a new protocol was not a main goal of this research and methods were roughly modelled from those summarised in the literature, the experimental design for this research can also contribute to assessing appropriate method development. First, the combined visual and chemical characterisation demonstrated that different techniques have different uses to quantify and characterise certain types and sizes of these materials. Furthermore, this confirmed that source cannot be inferred from one piece of information, whether this is chemical or visual. It is necessary to assess morphology, colour, size, and composition to determine the likely origin of the different particles. Additionally, two approaches that are not often part of routine protocols to date were incorporated – these included the use of an FTIR correction to account for error in reporting non-plastics (e.g. cellulose fibres) in MP estimates, and grain size fractionation to explore MP-sediment interactions. Furthermore, quality assurance tests were incorporated throughout the study, which are rarely reported but are necessary to validate protocols, improve transparency and transferability of methods, and promote inter-study conversations to advance research on method recommendations so that results can be compared.

Microplastic pollution is a priority in the political and science agenda, but this is still a new area of water research with many remaining questions. As researchers, it is necessary to produce the

empirical evidence necessary to inform government and regulation for measures that reduce global pressures on water supplies and the impact of anthropogenic littering on water quality. Therefore, this and similar studies are relevant to academia, government and industry worldwide, and can aid legislators, manufacturers, and other stakeholders in targeting research efforts on priority areas and developing effective monitoring and regulation strategies.

7.6 Future Work

Incisive understanding of MP pollution in WWTPs and rivers was generated here, and from this, the following areas that would be valuable for future research can be identified to address crucial questions that were not answered in this research:

1. Seasonality and spatial assessment should be considered for deeper insight on what controls small- vs. large scale and short- vs. long- term fluctuations.
2. As MP pollution appears controlled locally, a case-by-case assessment may be necessary to pinpoint main sources and identify solutions. This could mean generation of catchment-specific FTIR libraries of plastic materials used by local industries and households for accurate assessment and tracing of sources.
3. More compartments need to be incorporated in research studies, including biosolids and storm overflow channels in WWTPs, CSOs, and subsurface sediments and water in the rivers. Studying different liquid and solid compartments is relevant for mass balance analysis and to build a comprehensive picture of movement and retention of MPs in these systems.
4. The physical interactions between MPs and active biological fractions could provide a new area of research in WWTPs. For example, positive associations have been observed between bacteria and MPs as these materials can act as transport vectors for microorganisms (Virsek et al. 2017). These potential interactions should be explored for WWTP as harmful microorganisms may be transported from the WWTP to receiving waters, protected by the plastic.
5. Sinking of MPs in the natural environment by entrapment in phytoplankton aggregates (Long et al. 2015) could be relevant for understanding their removal in tertiary filtration processes, for example by examining their potential aggregation with biofilms. Currently, no studies have reported on these associations in WWTPs, representing a current knowledge gap in MPs research that could provide additional information to understand the impact of different treatment stages on MP loading to discharge.
6. Further information on exposure due to contact (e.g. ingestion or uptake via non-ingestion pathways, bioaccumulation, and trophic transfer) and ecotoxicity can be considered with the MP abundances in the Clyde catchment for risk assessment. Similar to abundance studies, current knowledge on exposure and ecotoxicity is based primarily on research on marine species, mainly fish, molluscs, and crustaceans and understanding of the effects of exposure remain incomplete. For example, efficient egestion has been observed in some species, and while detrimental effects have been reported at the individual and population, multiple studies have noted neutral or mixed responses (Anbunami and Kakkar 2018).

7. Evidence is missing on MP toxicity related to additives contained and adsorbed, some of which may be collected during passage through WWTPs (Wu et al. 2017).
8. The role of hydrology and sedimentation processes in rivers should be explored further. For example, recent studies suggest that MPs may behave like sediment so their retention and release could be predicted by transport models for sediment and low-density particles (Kooi et al. 2018). However, as observed here, different types of MPs may be associated with different size fractions, and thus future work should expand on the role of sediment-MP associations and biofouling mechanisms on MP sedimentation to inform model calibration.

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Appendix A Supplementary Electronic Material (Blair et al. 2017) and Addendum

Table A-1 Summary of microplastic surveys in freshwater and wastewater systems from 2011-2016; sorted by continent then year of publication (Blair et al. 2017

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
Africa	Lake Victoria, Tanzania	Biginagwa et al. 2015	Perch and Tilapia gut content	dissection of entire gastrointestinal tract from fish purchased from harbor market; NaOH digestion; FTIR-ATR	<5 mm - 0.5 mm (lowest threshold for FTIR-ATR analysis	Microplastics observed in 55% and 35% of tilapia and perch, respectively, but confirmed through FTIR-ATR only in 20% of each species due to size limitations of spectroscopic techniques.
America	Los Angeles River, Coyote Creek/San Gabriel River system, USA	Moore et al. 2011	surface, mid, and near-bottom water	manta trawl (mesh 1 mm), with a streambed sampler for mid and bottom samples; visual inspection with naked eye and dissecting microscope; sieving (mesh 4.75, 2.8, 1.0 mm)	1 - 4.75 mm (micro), >4.75 mm (macro)	Greatest abundances and densities observed during wet periods. Total microplastic abundances were 74 items per m^3 in Coyote Creek, 337 items per m^3 in San Gabriel, and 12,932 items per m^3 in L.A. River. Microplastics were 16x more abundant than macroplastics (3x more by weight). Most common debris type were foamed PS followed by pellets, hard plastic fragments, thin films, line, and whole items. Estimated yield was 2.33×10^9 plastic objects and particles over 72-hr period.
	Lake Huron, Canada/USA	Zbyszewski and Corcoran 2011	sediment	beach surveying for collection of visible debris with stainless steel trowel; FT-IR; SEM	<5 mm plastic pellets, >5 mm broken plastic, PS	In Lake Huron, a total of 3,209 pieces were found, consisting of 2,984 pellets, 108 fragments, and 117 pieces of styrofoam.
	Lakes Superior, Huron, and Erie, Canada/USA	Eriksen et al. 2013	surface water	manta trawl with (mesh 333 µm); sieving (0.355-0.999 mm, 1.00-4.749 mm, >4.75 mm); SEM/EDS	0.355-0.999 mm, 1.00-4.749 mm, >4.75 mm	Spatial variability observed across samples, ranging from ~450 to >450,000 items per km^2. Lake Erie (most populated) had the highest abundances. Average abundance was 43,157 items per km^2 for all samples. Most common debris were pellets and fragments. The smallest size class accounted for 81% of the total count. Most pieces are suspected to originate from consumer products, likely introduced by nearby urban effluent.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Great Lakes, USA	Rios Mendoza and Evans (abstract) 2013	n/a	n/a	n/a	n/a
	St. Lawrence River, Canada/USA	Castañeda et al. 2014	sediment	benthic grab (10 cm depth); sieving (500 µm); visual separation and identification under dissecting microscope; differential scanning calorimetry	0.40-2.16 mm (range of microbeads collected)	Microbeads found at 8 of 10 sites. Mean density was 13,759 $\pm$ 13,685 items per m^2 across all sites. Items were of various colours and sizes, their melting point suggested PE.
	Dunkirk, Fredonia, and Plattsburg WWTP in New York, USA	Chaskey et al. (poster) 2014	WWTP effluent	volume reduced sampling (pump and hose system); sieving during sampling (mesh 1, 0.355, 0.125 mm); H_2SO_4 and H_2O_2 digestion; visual inspection under dissecting microscope	<1 mm, 355 µm, 125 µm	Suspect plastic-like particles present in all WWTP effluent, discharged at rates of 109,556, 81,911, and 1,061,953 particles per day from Plattsburgh, Fredonia, and Dunkirk, respectively. Particle colour ranged from bright red and blue to opaque. Observed signs of erosion and UV-degradation.
	North Shore Channel, USA	Hoellein et al. (abstract) 2014	n/a	SEM; rRNA sequencing	0.3-5 mm	Microplastic concentrations higher downstream of WWTP relative to upstream. Most common debris were fragments and plastic fibres.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	North Shore Channel, USA	McCormick et al. 2014	surface water	neuston nets (mesh 333 µm); sieving (mesh 2 and 0.330 mm; H_2O_2 digestion; NaCl density separation; visual inspection under dissecting microscope; SEM; bacterial measurements (DNA extraction and sequencing); dissolved nutrients (SRP, NH_4, NOx)	2 mm-330 µm	Microplastics found in all samples. Microplastics and consituent concentrations were higher downstream of WWTP effluent discharge. Mean microplastic concentrations were of 1.94 $\pm$ 0.81 m^3 upstream and 17.93 $\pm$ 11.05 m^3 downstream. Foams and pellets were found downstream, but in lower concentrations than fragments and fibres. Extensive colonisation of microplastic pellets and fragments observed, mainly prokaryotic cells.
	Lakes Huron, Erie and St. Clair, Canada/USA	Zbyszewski et al. 2014	sediment	beach surveying for collection of visible debris with stainless steel trowel; FT-IR; SEM	<2 cm (styrofoam, pellets, plastic fragments), intact or near-intact debris	Microplastic abundances were 1,576 pieces in Lake Erie (603 pellets, 934 fragments, and 39 pieces of styrofoam) and 817 pieces in Lake St. Claire (110 pellets, 192 fragments, 234 pieces of styrofoam, and 281 intact or near-intact debris).
	Lake Ontario, Canada	Corcoran et al. 2015	sediment	beach surveying for collection of visible debris; Raman; box corer for lake bottom sediment samples; size fraction sieving (<0.5, 0.5-0.71, 0.71-0.85, 0.85-1, >1 mm); SPT; visual inspection under microscope; FTIR	<1 cm, 1-5 cm, >5 cm for visible samples; <5 mm (micro)	A total of 6,172 pieces collected from beach sites (pellets, fragments, intact items, and PS). Pellets showed composition of PE and PP. Most common pieces were 1-5 cm and white/translucent. Microplastics 0.5-3 mm in size found in bottom sediments (depth <8 cm), with PE accounting for the majority of microplastics. Higher abundances observed in sediment collected at site near the centre of the lake vs site near the outlet.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Pattsburgh WWTP in New York, USA	Buksa and Niekerewicz 2016	WWTP effluent	volume reduced sampling; sieving (mesh 1, 0.355, 0.125 mm); KOH and H_2O_2 digestion; visual inspection under dissecting microscope; FTIR	<1 mm, 355 µm, 125 µm	Most common type were fibres. Least common type was foam. Most plastics were 0.125 and 0.355 micron. High flow rate correlated with high pellet abundance and low flow rate correlated with high film abundance.
	Lake Ontario, Canada	Ballent et al. 2016	nearshore, tributary, and beach sediment	combination of sediment trap, core and grab sampling techniques; sieving (mesh 5.6, 2.0, 0.063 mm); density separation with SPT; visual inspection with stereo microscope; FT-Raman	according to size fraction sieving	Total microplastic count was 6,331 microplastic particles. Average abundance was 760 items per kg dry sediment (ranging from 20 to 27,830 items per kg dry sediment). Abundances for the different sediment locations as follows: nearshore > tributary > beach. Most common types were fibres and fragments (<2.0 mm fraction), and fragments and beads (>2 mm fraction).
	WWTPs in Southern California, USA	Carr et al. 2016	tertiary and secondary effluent	sampling with sieving (400, 180, 45, 20 µm); surface filtering for skimming water surface at final outfall; centrifuge; digestion with bleach; visual inspection under microscope; FT-IR	according to size fraction sieving	Tertiary treatment plant found effective in removing microplastics via skimming and settling processes. In secondary plants, 373 particles (>90% blue PE fragments) found in effluent (equivalent to yield of 1 microplastic per 1.14 x 103 L). Most common type was blue PE similar to that of toothpaste.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Raritan River, USA	Estahbanati and Fahrenfeld 2016	water	plankton nets (mesh 153 µm), H^2O^2 digestion with iron (II) catalyst; NaCl density separation; visual inspection under stereo microscope	500-2000 µm, 250-500 µm, 125-250 µm, 63-125 µm	Microplastics 250-500 µm and 125-250 µm increased downstream. Secondary microplastics more abundant in all but smaller size class. Primary microplastics increased downstream in all but larger size class. Moderate correlation between distance and concentration.
	Palisades Reservoir and Snake River, USA	McDevitt et al. 2016	water	Bulk sampling; vacuum filtration (0.45 um); visual inspection under stereo microscope and compound light microscope	n/a	Suspected microplastics observed in 72% of 11 samples, 25% were films.
Asia	Lake Hovsgol, Mongolia	Free et al. 2014	shoreline debris; surface water	shoreline surveying for collection of visible pieces; manta trawl (mesh 333 µm); sieving; H_2O_2 digestion; density separation; visual inspection under light microscope	0.355-0.999 mm, 1.00-4.749 mm, >4.75 mm	Total plastic count was 409 items in shoreline (77% were macroplastics). Pelagic microplastics averaged 20,264 items per km^2 (ranging from 997 to 44,435 items per km^2). Most common types were fragments, films, and lines/fibres.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Lake Taihu, China	Su et al. 2016	water, sediment, organisms	plankton nets (mesh 333 µm); bulk water sampling with steel sampler; grab sampling for sediment; bottom fauna trawl sampling; H2O2 digestion; NaCl density separation; visual inspection under stereo microscope; micro FT-IR; SEM/EDS	333-5000 µm	Microplastic abundances were 0.01 x 10^6 to 6.8 x 10^6 items per km^2 in plankton nets, 3.4 to 25.8 items per L in surface water, and 11.0 to 234.6 items per kg dry weight in sediment. Most common type were fibres (48-84%). Most common colours were blue in plankton net and surface water, and white and transparent in sediment and organisms. Most common sizes were 100-1000 µm in surface water, sediment, and organisms, and 333-5000 in plankton nets.
	Tibet plateau, China	Zhang et al. 2016	lakeshore sediment	lakeshore sediment collected with shovel (20x20 cm^2 quadrats); sieving (mesh 1 mm); HCO_2K density separation;visual inspection with stereo microscope; Raman; SEM	<0.5 mm, 0.5-1 mm, 1-5 mm	Microplastics detected in 6 of 7 sites. Abundances were 8 $\pm$ 14 to 563 $\pm$ 1,219 items m^2. Most common sizes were 1-5 mm. Polymers identified included: PE, PP, PS, PET, PVC.
Europe	Lake Geneva, Switzerland	Faure et al. 2012	surface water; sediment; fish and birds	sand sieving and beach surveying for collection of coarse fragments; manta trawl (mesh 300 µm); collection of gut content from fauna; visual inspection under stereo microscope	<2 mm, 2-5 mm (sediment); <5 mm, > 5mm (water)	In sand samples, 1-7 fragments found. Most common type was PS. In water samples, densities were 7,649 items per km^2 and 48,146 items per km^2 for macro- and microplastics, respectively (data from only one sample). No ingested plastics were observed in guts of fauna.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Jade System, Southern North Sea, Germany	Dubaish and Liebezeit 2013	surface waters; effluent (paper recycling plant)	grab sampling (20 cm depth); sieving (mesh 40µm) during sampling; visual inspection under dissecting microscope	not specified	In Jade system, mean abundances were 64 ± 194 (granules) and 88 ± 82 (fibres) items per L. In sewage effluent, abundances ranged from 23 to 52 (granules), 23 to 25 (fragments), and 12-41 (fibres) items per L. Estimated average annual yield was $9x10^8$ particles from the treatment plant.
	Lake Garda, Italy	Imhof et al. 2013	sediment	random grid sampling; density separation; Raman; SEM	9-500 µm, 500 µm-1 mm, 1-5 mm, >5mm	In the northern shore, concentrations were 483 ± 236 (macroplastic) and 1,108 ± 983 (microplastic) particles per m^2. In the southern shore, concentrations were 8.3 (macroplastic; found only in one sample) and 108 ± 55 (microplastic) particles per m^2.
	Lagoon of Venice, Italy	Vianello et al. 2013	sediment	box corer sampling; NaCl density separation; micro-FT-IR; SEM	<1 mm (small micro, S-MPPs)	Total abundances ranged from 2,175 to 672 particles per kg^1. Polymers identified included: PE, PP, PEP, Pest, PAN, PS, Alkyd, PVC, PVOH, and Polyamide. Most common types were PE and PP (>82%). Irregular fragments, fibres, films, and pellets/granules, respectively, observed in 87%, 10%, 2%, and 1% of all stations. Most common sizes were 30-500 µm (93%). High correlation between total S-MPPs concentrations and finer sediment fraction and metal pollution index. Observed signs of degradation.
	River Dommel, Netherlands	Besseling et al. (abstract) 2014	n/a	spatially and temporally explicit model based on advective transport, homo- and hetero-aggregation, sedimentation-resuspension, polymer degradation and burial.	nano-, micro-, and millimetre (NMM) sized particles; size range for each class not specified	Particle size, biofilm formation and water turbulence affected fate and retention of NMM sized polymer particles and the positioning of the accumulation hot spots along the river.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Danube river, Austria/Slovakia	Lechner et al. 2014	surface water	driftnets (mesh 500 µm); density separation; visual sorting and inspection with naked eye	<2 mm (micro), 2-20mm (meso)	Average plastic load was 316.8 $\pm$ 4,664.6 items per 1000m^3 (79.4% industrial, 20.6% others) by density, or 4.8 $\pm$ 24.2 g per 1000m^3 (29.7% industrial, 70.3% others) by mass.
	Langeviksverket WWTP in Lysekil, Sweden	Magnusson and Nóren 2014	incoming and effluent water; sludge; recipient water	Ruttner sampler for effluent water; filtering (mesh 300 µm) during sampling; towing of zooplankton nets (mesh 300 µm) at 20, 50, and 200 m downstream of effluent tube; visual inspection under stereo microscope; FT-IR	$\geq$300 µm	In incoming water, mean concentration was 15,000 particles per m^3, equivalent to inflow of 3,200,000 particles per hour. More than 99% were retained in sludge. Effluent water discharged 1,770 particles per hour. In recipient water, mean concentrations were of 1.1-1.8 particles per m^3 were found in recipient water compared to 0.45 m^3 in reference site. Higher concentrations observed near effluent tube vs 200 m downstream. Fibres retained to higher degree in treatment.
	Thames river, UK	Morritt et al. 2014	surface water	GPS tracked fyke nets (mesh size not defined); direct counts	not specified	A total of 8,490 plastic items collected. Seven main categories identified. General plastics made up 20-25% of total litter in all sites, while wrappers and containers accounted for 21-28%. No major trends observed moving from upstream to downstream sites, but higher number of items observed near sewage treatment outflows.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Urban Paris sites, France	Dris et al. 2015	atmospheric fallout; settled and treated wastewater; surface water	funnel + bottle for collection of atmospheric fallout; 24-hr automatic sampler for collection of wastewater; plankton net (80 µm) and manta trawls (330 µm) for collection of surface water; visual inspection under stereo microscope; Histolab software for fibre quantification	100-500 µm, 500-1000 µm, 1000-5000 µm	In atmospheric fallout, 29 to 280 particles per m^2 per day observed. In wastewater, fibre concentrations ranged from 260 x 10^3 to 320 x 10^3 particles per m^3. Treatment decreased concentrations and sizes. Generally, most common type were fibres. No upstream to downstream evolution was observed.
	Rhine River, Germany	Mani et al. 2015	surface water	manta trawl (333 µm); sieving (mesh 5.0, 1.0, 0.3 mm); NaCl density separation; enzymatic digestion	according to size fraction sieving	Microplastics found in all samples, on average 892,777 particles per km^2. Load increased downstream, with peak concentrations in the metropolitan area. Differences along the river reflected effect of sources, hydrological dynamics, and potential sinks. Most common debris were opaque spherules and PS.
	Lakes Bolsena and Chiusi, Italy	Fischer et al. 2016	surface water; sediment	manta trawl for collection of surface water; collection of top 3 cm sediment (0.25 m^2 quadrat); sieving (mesh 5.0, 1.0, 0.5,0.3 mm); NaCl density separation; HCl digestion; staining with lipophilic dye; UV microscope; SEM	>5 mm, 1.0-5.0mm, 0.5-1.0 mm, 0.3-0.5 mm, <0.3 mm	In surface water, abundances were 2.68 to 3.36 (Lake Chiusi) and 0.82 to 4.42 particles (Lake Bolsena) m^3. In sediment, abundances were 112 (Lake Bolsena) and 234 (Lake Chiusi) particles per kg dry weight. Higher fibre concentrations observed in Lake Chiusi vs Lake Bolsena, attributed to sediment grain distribution.

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	WWTW in Glasgow, UK	Murphy et al. 2016	wastewater (influent after screens, grit and grease effluent, primary effluent, and final effluent), grit and grease, sludge cake	10-L steel buckets for collection of 30-50L (pooled) wastewater; visual inspection under dissecting microscope; FT-IR	n/a	A total of 430 plastic items observed, 8 of which were macroplastic. Abundances for the different samples were as follows: liquid fraction (n = 303) > solid fraction (n = 79) > sludge cake (n = 48). Secondary treatment found efficient in removing microplastics via grit and grease removal stage. Average abundances were 15.70 $\pm$ 5.23 (influent), 0.25 $\pm$ 0.04 (effluent) items per L, and 19.67 + 4.51 (grit and grease) items per 2.5 g. Microbeads observed only in grit and grease.
	Rivers Elbe, Mosel, Neckar, and Rhine, Germany	Wagner et al. 2014	sediment	density separation; visual inspection	<5 mm	Concentrations of 34-64 items per kg^1 dy weight were measured, and River Rhine had the highest load. Fragments accounted for 60% of total microplastics, and the remainder were fibres.
	Lakes Geneva, Constance, Neuchâtel, Maggiore, Zurich, and Brienz , Switzerland	Faure et al. 2015	surface water; sediment; fish and birds	spatial sampling for collection of beach sediments; NaCl density separation; manta trawl (mesh 300 µm) for water samples; collection of gut content from fauna; visual sorting and inspection under dissecting microscope for larger fragments; H_2O_2 digestion; FT-IR; mass spectrometry (gas and liquid chromatography)	>5 mm (macro), >1 mm (large micro), > 300 µm (small micro)	Microplastics found in all beach sediments and surface water samples. Macroplastics were also observed. In beach samples, average densities were 1,300 $\pm$ 2,000 (microplastic; ranging from 20-7,200 items per m^2) and 90 $\pm$ 250 (macroplastic; ranging from 0-150,000 items per m^2). In surface water, average densities were 91,000 $\pm$ 120,000 (microplastic) and 1,800 $\pm$ 3,100 (macroplastic) items per km^2 . In rivers, average densities were 790 $\pm$ 1,600 (microplastic) and 1.9 $\pm$ 3.5 (macroplastic) items per h^1. Pellets were less abundant but had a higher mass proportion than foams and fibres. Polymers identified included: PE (62%), PP (15%), and PS (12%). Ingested plastics observed in 7.5% of fish and 8 of 9 birds (mean of 4.3 $\pm$ 2.6 items per bird).

Continent	Location	Reference	Sample Medium	Materials	Size Class	Main Findings
	Rivers Rhine and Main, Germany	Klein et al. 2015	sediment	composite sampling of wet sediment; sieving (mesh size >10 mm) during sampling; sieving (mesh 63, 200, 630 µm); NaCl density separation; H_2O_2 and H_2SO_4 digestion; visual inspection with naked eye for pieces >630 µm and under binocular microscope for pieces 63-630 µm; FT-IR	630-5000 µm, 200-630 µm, 63-200 µm (discarded particles <63 µm)	Abundances ranged from 228 to 3,763 (Rhine) and 786 to 1,368 (Main) items per kg^1. Most abundant sizes were 630-5,000 µm by weight and 63-200 µm by count. Most common types were spheres and fibres in lower size classes and fragments in the 630-5,000 µm size class. Most common polymers were PE and PP (>50%) by weight and PS by count.
Worldwide	Multiple	Browne et al. 2011	sediment; effluent	grab sampling for collection of effluent; NaCl density separation; FT-IR	n/a	Abundances ranged from 2 to 31 fibres per 250 mL of sediment. Most common types were Pest (56%), followed by acrylic (23%), PP (7%), PE (6%), and polyamide fibres (3%). Abundances positively correlated with population density. Disposal sites contained >250% more microplastics than reference sites. Effluent contained at least 1 particle of microplastic per litre, and again Pest was most common.

Table A-2 Summary of methodological approaches used by freshwater river studies

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
Moore et al. 2011	Los Angeles River, Coyote Creek/San Gabriel River system, San Francisco, USA	November and December 28, 2004 for wet period, April 11, 2005 for dry period	Surface, mid, and near-bottom water	Handnets, manta trawl, streambed sampler, rectangular net (mesh sizes 0.333-0.88 mm)	Size class through 4.75, 2.8, 1.0 mm Tyler sieves; oven drying 65°C	Naked eye and dissecting microscope		Fragment, foam, pellet, line, film	1.0-4.75, >4.75
Castañeda et al. 2014	St. Lawrence River, Quebec, Canada	September 2013	Sediment	Benthic grabs ~10-15 cm depth	Sieving through 500 µm and preserved in ethanol	Dissecting microscope; differential scanning calorimetry		Microbead	0.5
Lechner et al. 2014	Danube river, Austria	2010, 2012	Surface water	Stationary driftnets (mesh 500 µm);	Water bath and density separation with visual sorting	Visual		Pellet, spherule, flake, other	<2, 2-20
McCormick et al. 2014	North Shore Channel, Chicago, USA	September 13, 2013	Surface water	Neuston net tows (mesh 300 µm	Sieving through 2 and 0.330 mm mesh, oven drying 75°C; H_2O_2 digestion; NaCl density separation;	Dissecting microscope; SEM	Bacterial measurements (DNA extraction and sequencing); dissolved nutrients (SRP, NH4, NOx)	Fragment, pellet, foam, fiber	0.33-2.0
Wagner et al. 2014	Rivers Elbe, Mosel, Neckar, and Rhine, Germany	Not specified	Sediment	Not specified	Density separation	Visual		Not specified	<5.0

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
Dris et al. 2015	Urban Paris sites, France	April 23 and May 14, 2014 for plankton nets, July 17, 2014 for manta trawl	Surface water	Plankton net (mesh 80 µm) and manta trawls (mesh 330 µm)	Filtration only	Stereo microscope; Histolab software for fibre quantification	Settled and treated wastewater, atmospheric fallout	Not specified	0.1-0.5, 0.5-1.0, 1.0-5.0
Faure et al. 2015	Lakes Geneva, Constance, Neuchâtel, Maggiore, Zurich, and Brienz, Switzerland, and a few rivers	July-October 2013 for lakes, after October 2013 for rivers	Surface water, sediment, fauna	Spatial bulk sampling of beach sediments, manta trawl (mesh 300 µm) for water samples;	NaCl density separation; H_2O_2 digestion	Dissecting microscope; FTIR; mass spectrometry (gas and liquid chromatography)	Gut content from fauna; adsorbed micropollutants and toxic additives	Fragment, pellet, cosmetic bead, line, fibre, film, foam	>0.3, >1.0, >5.0
Klein et al. 2015	Rivers Rhine and Main, Germany	Not specified	Sediment	Composite sampling of 3-4 kg wet sediment	Oven drying 50°C; size class fractionation (63, 200, 630 µm); NaCl density separation; H_2O_2 and H_2SO_4 digestion	Naked eye for >630 µm and binocular microscope for pieces 63-630 µm; FTIR-ATR		Pellet, sphere, fragment, fiber	0.063-0.2, 0.2-0.63, 0.63-5.0

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
Mani et al. 2015	Rhine River, Germany	June-July 2014	Surface water	Manta trawl net (mesh 333 µm);	Wet fractionation (5.0, 1.0, 0.3 mm); NaCl density separation; SDS enzymatic digestion	Stereo microscope; FTIR-ATR		Fragment, fibre, spherule opaque, spherule transparent, other (foam, foil, pellet)	0.3-5.0
Zhang et al. 2015	Three Gorges Dam (Yangtze River and four tributaries), China	September 23, 2014	Surface water	Trawl nets (mesh 112 µm)	Wet sieving through 1.6 mm, separating funnel; oven-drying of floating debris 60°C	Light microscope; FTIR-ATR		Sheet, line, foam, fragment	0.112-0.3, 0.3-0.5, 0.5-1.6, 1.6-5.0
Baldwin et al. 2016	Great Lake tributaries, USA	April 2014-April 2015	Surface water	Neuston net (mesh 333 µm	Size class fractionation (4.75, 1.0, 0.355 mm); H_2O_2 digestion with iron (II) catalyst, final sieving through 123 µm	Dissecting microscope	Urban characteristics (land cover, population density, wastewater effluent); hydrology	Fragment, pellet/bead, line/fibre, film, foam	0.999-0.355, 4.749-1.0, >4.75,

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
Ballent et al. 2016	Lake Ontario and tributaries, Canada	June 2015	Sediment	Shipek grab	Oven drying 70°C; size class fractionation (5.6, 2.0, 0.063 mm); SPT density separation; magnetic stirring and separatory funnel	Stereo microscope; FT-Raman and X-ray fluorescence spectroscopy	Population density; plastics-related industries	Fibre, fragment, spherical bead	<2.0, >2.0
Estahbanati and Fahrenfeld 2016	Raritan River, USA	October-November 2015	Surface water	Fixed plankton nets (mesh 153 µm) (1.3-3.5 m^3)	H_2O_2 digestion with iron (II) catalyst; NaCl density separation	Stereo microscope		Not specified	0.063-0.125, 0.125-0.25, 0.25-0.5, 0.5-2.0, 1.0-2.0, 2.0-4.0
Horton et al. 2016	River Thames tributaries (River Leach, River Lambourn, The Cut), United Kingdom	August 28-September 3, 2014	Sediment	Bulk sampling across 3 m transect parallel to bank	Oven drying 80°C; visual extraction of all sample; ZnCl density separation, oven drying of fitlers 60°C	Light microscope; Raman	Estimation of population equivalents	Fragment, fibre, film	

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
McCormick et al. 2016	Illinois and Indiana, USA (nine rivers all receiving WWTP discharge)	July 10-October 13, 2014 (single date for each)	Surface water	Neuston net (mesh 333 µm	Wet sieving (4.75 mm and 330 um); oven drying 75°C; H_2O_2 digestion with iron (II) catalyst; NaCl density separation	Dissecting microscope; py-GCMS	Bacterial assemblage; dissolved nutrients	Fiber, film, fragment, pellet, foam	0.33-4.75
Leslie et al. 2017	Rivers Meuse and Rhine, Germany	Between 2012 and 2013	Surface water suspended particulate matter (SPM)	Continuous centrifugation system for SPM	NaCl density separation	Light microscope; FTIR in transmission mode	WWTP water; canal water and sediment; marine sediment and biota	Fibre, sphere, foil	<0.3, >0.3
Miller et al. 2017	Hudson River, New York, USA	June and October 2016	Surface water	Grab sampling (1L)	Filtration only	Stereo microscope; µ-FTIR		Fibre	By length: 0.1-1.5, 1.6-3.2, 3.3-9.6
Vermaire et al. 2017	Ottawa River, Canada	Fall 2015 for sediment and Summer 2016 for water	Surface water and sediment	Grab sampling (100L) and manta trawl (mesh 100 um; 100,000L) for water and Ekman grab for sediment	Oven drying 100°C, NaCl density separation, H_2O_2 digestion	Stereo microscope	WWTP effluent	Not specified	>0.1

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
Wang et al. 2017	Beijian River littoral zone, China	March 2015	Sediment	Bulk sampling	NaCl density separation; ultrasonic bath, oven drying of filter 50°C	Digital handheld microscope; µ-FTIR, SEM-EDS and ICP-MS for metals	Metals	Not specified	Not specified
Wang et al. 2017	Hangjiang River and Yangtze River, and lakes in Wuhan, China	April 2016	Surface water	Teflon pump and 50 µm sieve (20L)	H_2O_2 digestion with iron (II) catalyst	Stereo microscope; FTIR; SEM		Fiber, granule, film, pellet	<0.5-1.0, <1.0-2.0, <2.0-3.0, 3.0-4.0, 4.0-5.0
Zhang et al. 2017	Xiangxi River (Three Gorges Dam), China	April, July, and October 2015 for water and January 2016 for sediment	Surface water and sediment	Trawl nets (mesh 112 µm) for water and Peterson grab for sediment		Stereo microscope; Raman	Fish got content; phytoplankton and zooplankton; water parameters (DO, pH, temperature, EC, secchi depth, water level, TP, TN, NH_4-N, NO_3-N	Sheet, fragment, line, foam	0.112-0.5, 0.5-1.0, 1.0-5.0
Di and Wang 2018	Yangtze River, China	August 2016	Surface water and sediment	Teflon pump and 48 µm sieve (25L) for water and Van Veen grab for sediment	H_2O_2 digestion with iron (II) catalyst; NaCl + NaI sequential density separation, oven drying of filter 50°C	Dissecting microscope; Raman; SEM	Co-pollutants on MP surfaces	Fiber, fragment, pellet, film, styrofoam	<0.5, 0.5-1.0, 1.0-2.0, 2.0-3.0, 3.0-4.0, 4.0-5.0

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
Hurley et al. 2018	Irwell River and Mersey River catchments, United Kingdom	Between April and July 2015 (preflood) and May and Mid July 2016 (postflood)	Sediment	Cylinder resuspension apparatus	NaCl, NaI, and sewater density separation, oven drying filter 50°C	Stereo microscope; FTIR-ATR		Fragment, fibre, microbead, other	0.063-5.0
Nel et al. 2018	Bloukrans River, South Africa	February 16-18 and July 14-17, 2016	Sediment	Bulk sampling	Oven drying 50°C, saline density separation	Dissecting microscope	Chironomids; environmental parameters (river flow, water depth, channel width, substrate embeddedness, sediment, organic matter	Not specified	0.063-5.0
Peng et al. 2018	Shanghai rivers and tidal flat, China	July and August 2016	Sediment	Bulk sampling	Oven drying 70°C; NaCl density separation	microscope; μ-FTIR		Sphere, fiber, fragment	Not specified
Rodrigues et al. 2018	Antua River, Portugal	March and October 2016	Surface water and sediment	Pump with 0.055 mesh for water (1.2 m^3) and Van Veen grab for sediment	Oven drying 90°C, ZnCl density separation; H_2O_2 digestion with iron (II) catalyst; oven drying filter 40°C	Stereo microscope; FTIR-ATR	Conductivity, oxygen saturation, temperature, pH, flow velocity	Fragment, pellet, film, foam, fibre	0.055-5.0

Study	Location	Sampling Period	Sample Medium	Sampling Approach	Extraction Methods	Characterisation Methods	Other Measurements	Classifications	Size Classes (mm)
Schmidt et al. 2018	Teltow Canal, Berlin, Germany	May and August 2015	Surface water	Composite grab sampling (83.61-132.01 L) and on -site filter through 20 µm mesh	Oven drying 70°C; H_2O_2 digestion	Short-wave infrared (SWIR) imaging spectrometer		Not specified	>0.45
Kataoka et al. 2019	29 rivers in Japan	August 2015 - May 2018	Surface water	Plankton net (mesh 335 µm)	NaCl density separation	Stereo microscope; FTIR	Basin characteristics (population density, urban and agricultural area, urban/agricultural area:basin area ratios); water quality parameters (pH, BOD, TN, TP)	Fragment, primary	Not specified
Shuri et al. 2019	Atoyac River basin, Mexico	Zahuapan River, Atoyac River, Confluence zone, Valquesillo Dam (Atoyac River basin), Mexico	Sediment	Trowel for rivers and Van Veen grab for dam	Oven drying 40°C; H_2O_2 digestion; ZnCl density separation	Stereo microscope, SEM-EDS		Film, fragment, fiber, pellet	Not specified

Appendix B Study sites

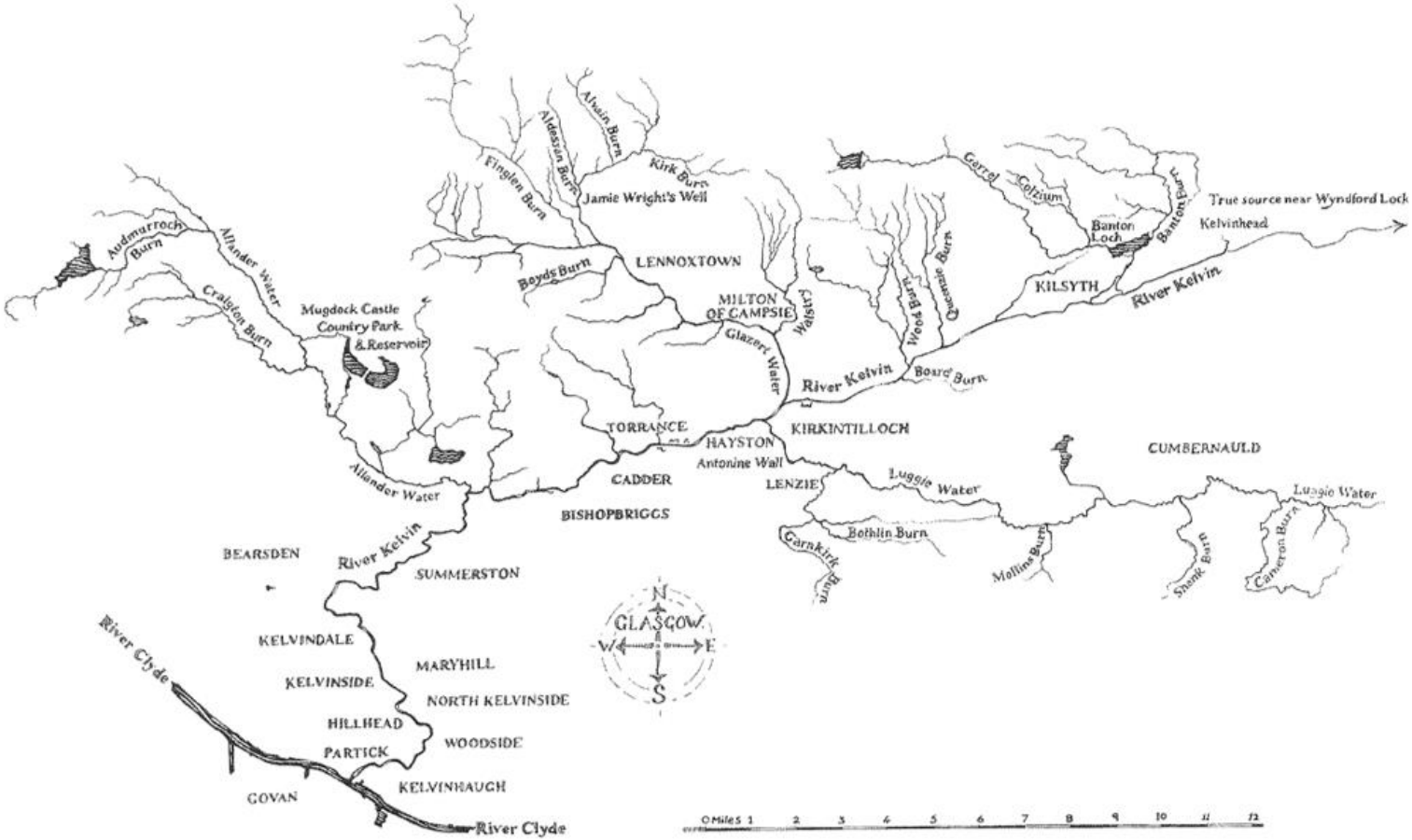


Figure B-1 Map for the River Kelvin and its tributaries published in Matheson 2000

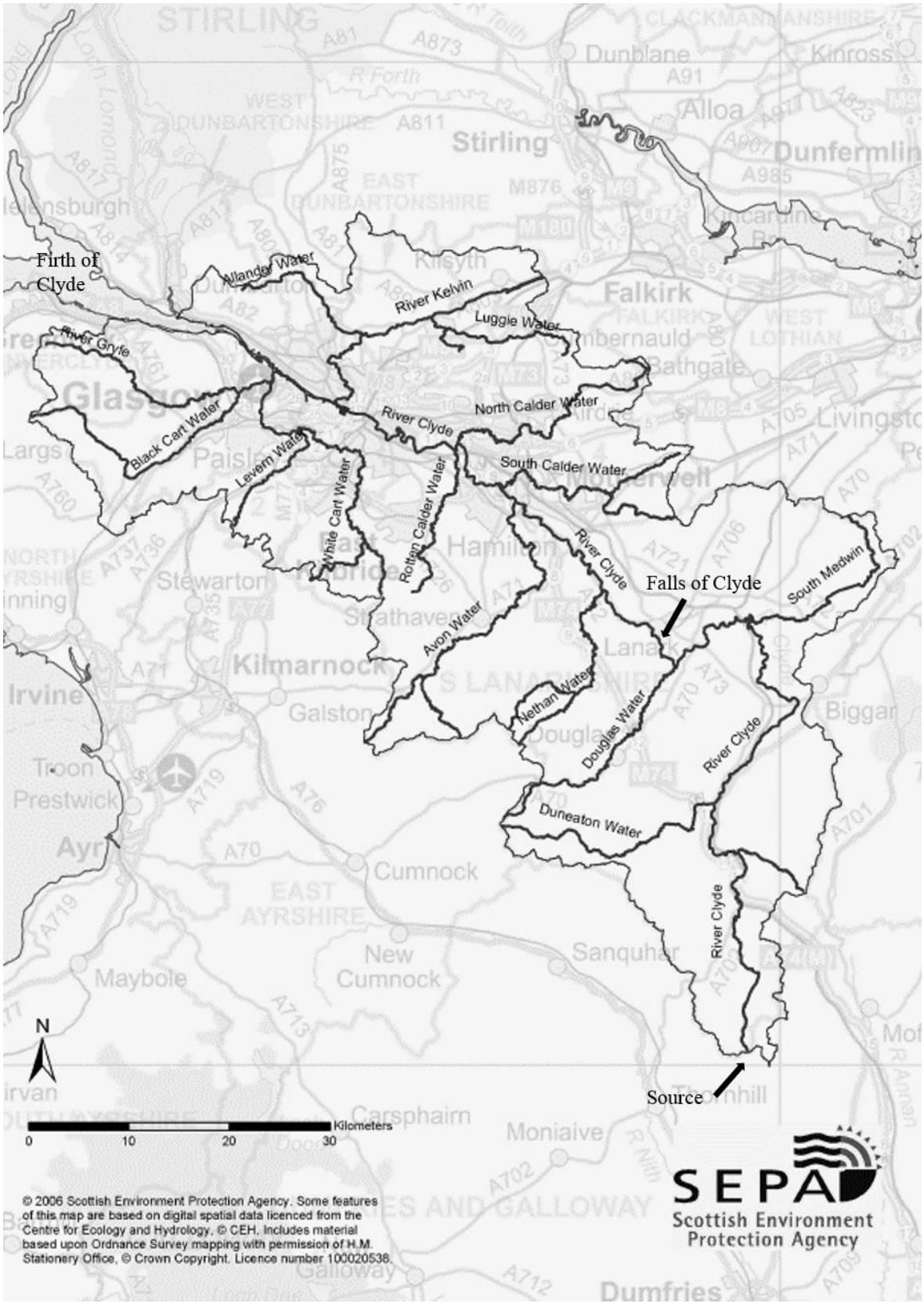


Figure B-2 Map of the River Clyde and tributaries from its source in South Lanark to its discharge into the Firth of Clyde. Base map published in © Ioris 2006.

Table B-1 Summary of pre-selected wastewater treatment plants (WWTP) discussed in conjunction with Scottish Water and SEPA

WWTP	Urban	Freshwater Recipient	Distance to Lab (min)	Size (p.e.)	Secondary Treatment	Tertiary Treatment	Sludge Treatment	Third-party Imports	Other Interest
Dalderse	**Yes**	**Yes**	**40**	78000	Activated sludge	No	Treated on site by anaerobic digestion.	Yes	Part of CIP
Dalmarnock	**Yes**	**Yes**	**18**	260000	Activated sludge	No	Glasgow Sludge main - Daldowie PFI	No	
Daldowie	**Yes**	**Yes**	**19**	184500	Activated sludge	Nitrfying plastic filters	Glasgow Sludge main - Daldowie PFI	Yes	Part of CIP, ongoing testing for P removal with Fe
Hamilton	**Yes**	**Yes**	**26**	50000	Activated sludge	No	Shieldhall - Daldowie PFI	No	
Bothwellbank	**Yes**	**Yes**	**29**	20327	Activated sludge	No	Shieldhall - Daldowie PFI	No	
Phillipshill	**Yes**	**Yes**	**27**	45000	Activated sludge	No	Shieldhall - Daldowie PFI	No	
Laighpark (Paisley)	**Yes**	**No**	**18**	240000	Activated sludge	No	Shieldhall - Daldowie PFI	No	Recent technology upgrade for improved real-time monitoring
Shieldhall	**Yes**	**No**	**12**	574000	Activated sludge	No	Shieldhall - Daldowie PFI	Yes	One of largest operated by Scottish Water

Abbreviations: **p.e.**, population equivalent; **PFI**, public finance initiative; **CIP**, chemical investigations programme

Appendix C Materials and methods

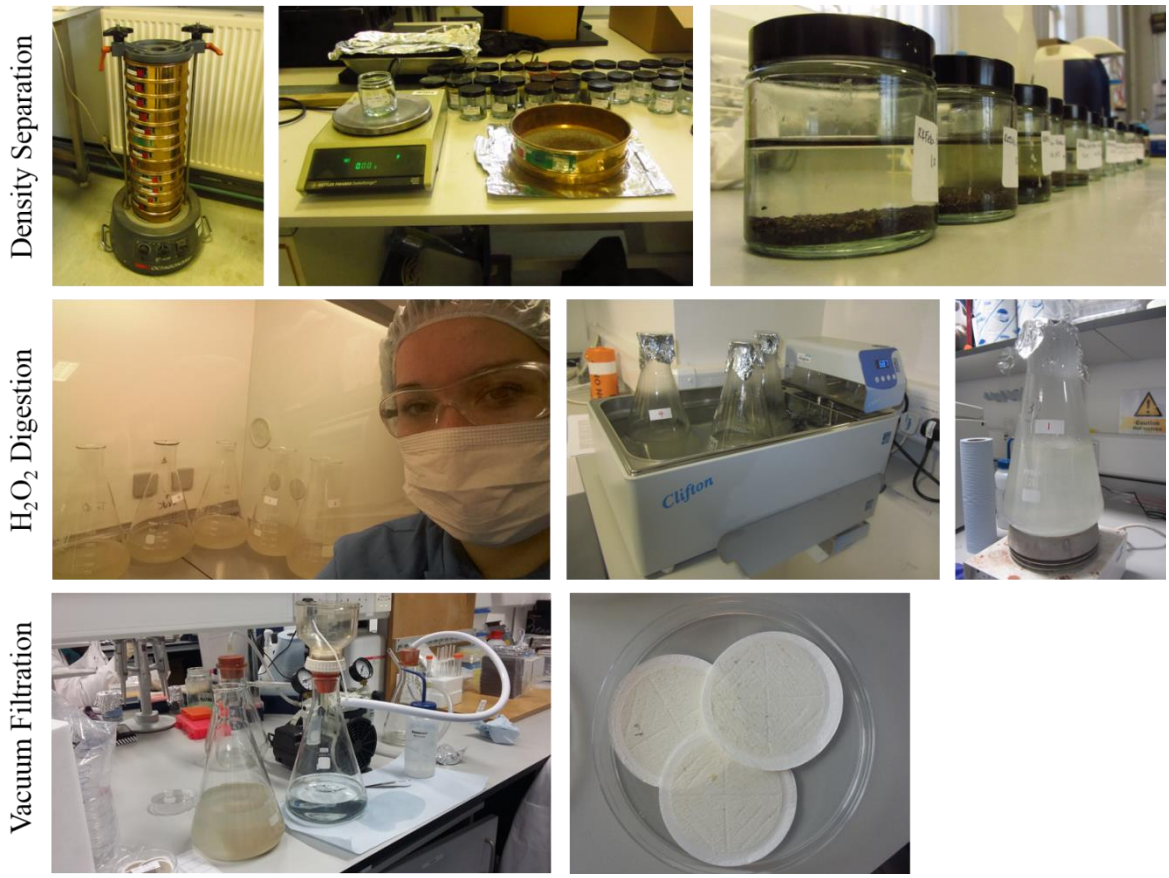


Figure C-1 Sample lab setup for extraction of MPs by density separation (top; sediment), hydrogen peroxide (H_2O_2) oxidation (middle; wastewater), and vacuum filtration (bottom; density separation supernatant, digested wastewater, and untreated surface water)

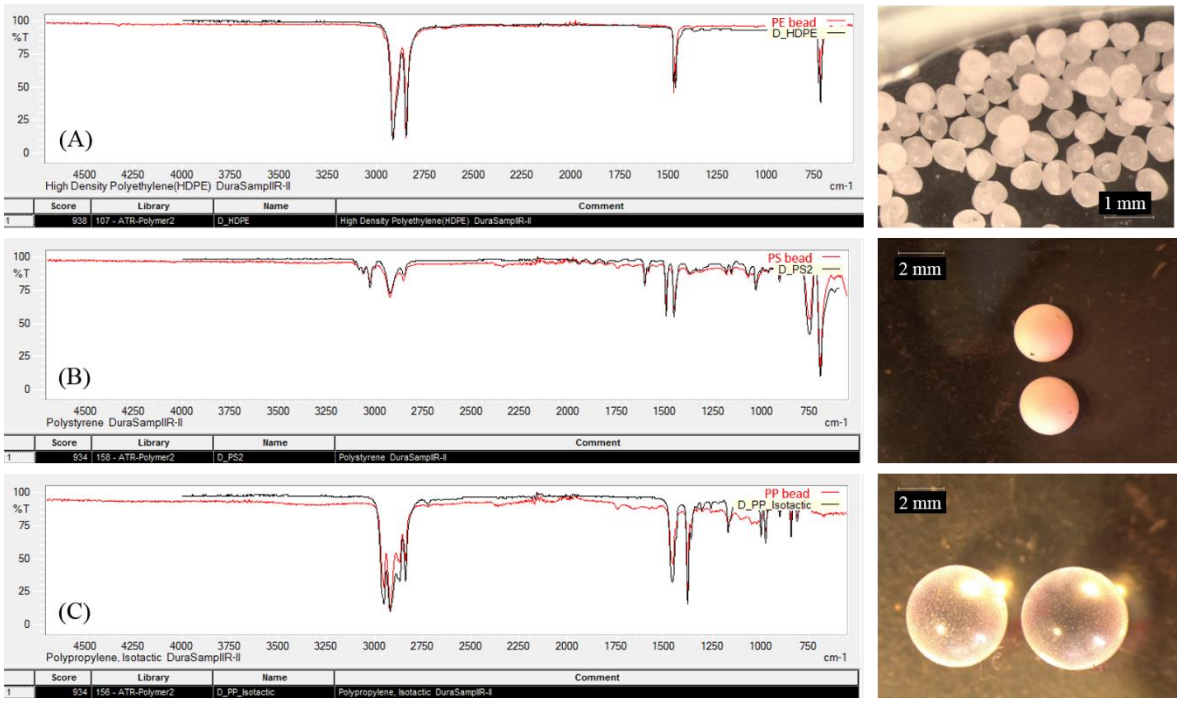


Figure C-2 Chemical and visual characteristics of (A) polyethylene, (B) polystyrene, and (C) polypropylene bead standards used in this project. Standards were purchased from Cospheric LLC, Santa Barbara, CA.

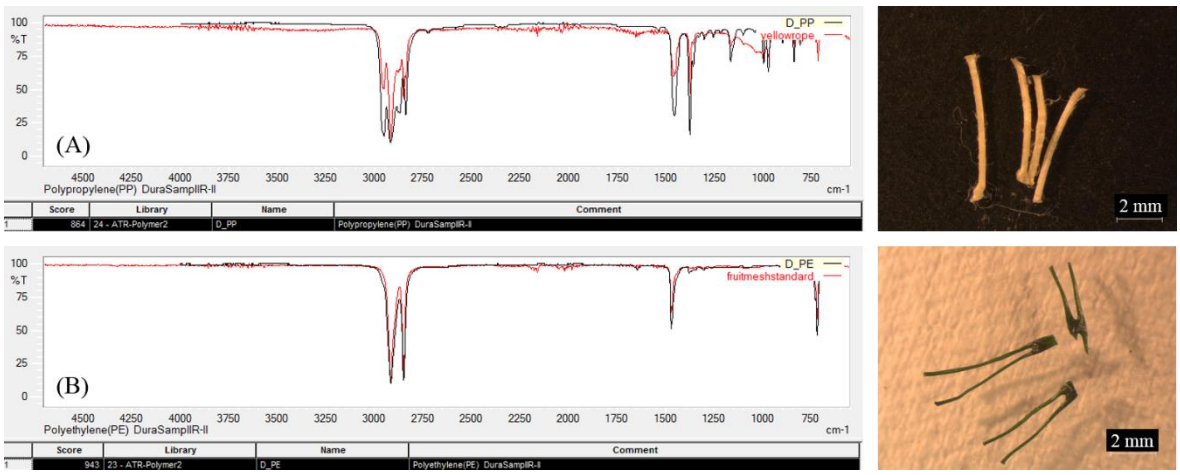


Figure C-3 Chemical and visual characteristics of (A) polypropylene and (B) polyethylene fibre-like standards used in this project. Standards were created in the lab.

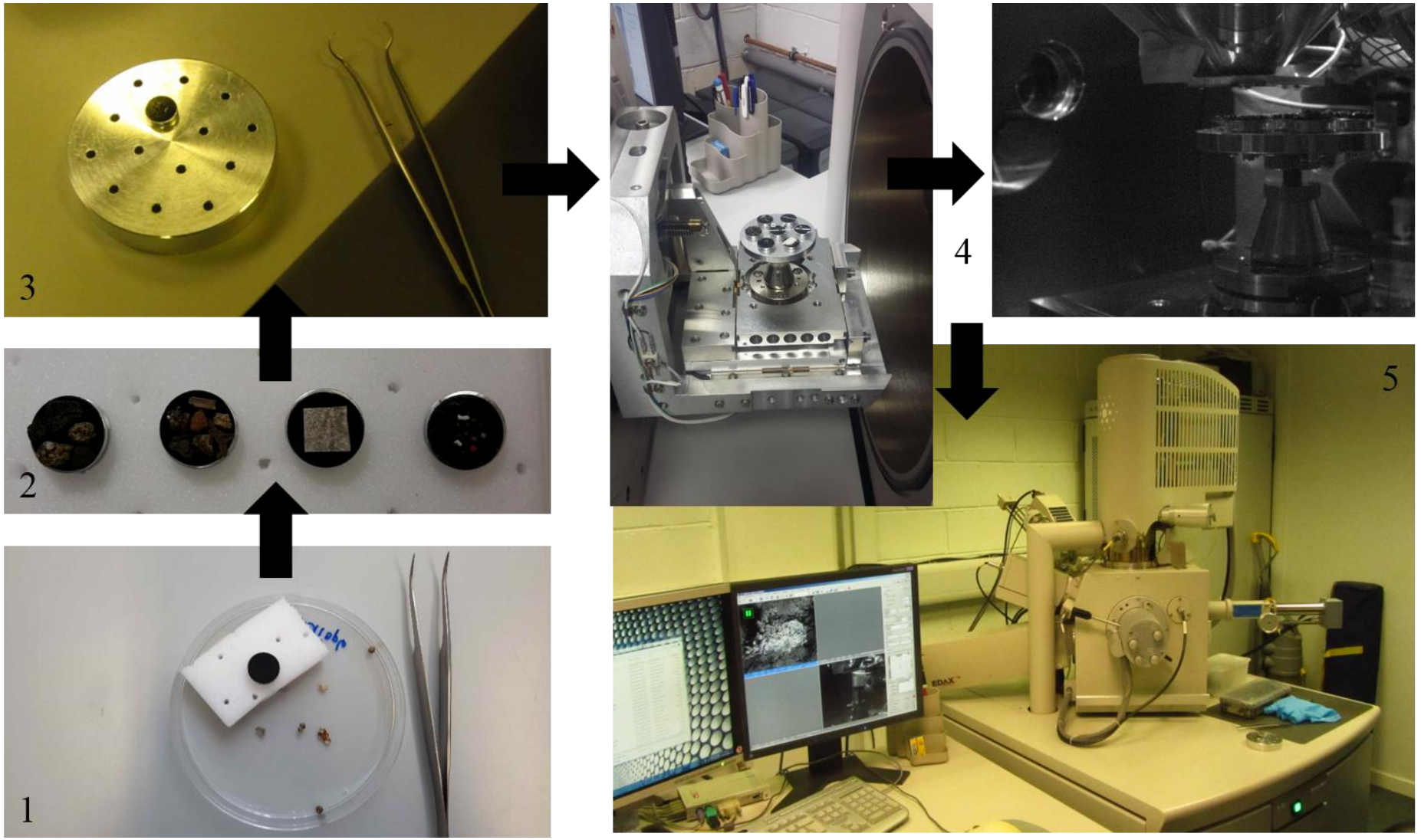


Figure C-4 Sample set up for SEM-EDS analysis of suspected MPs

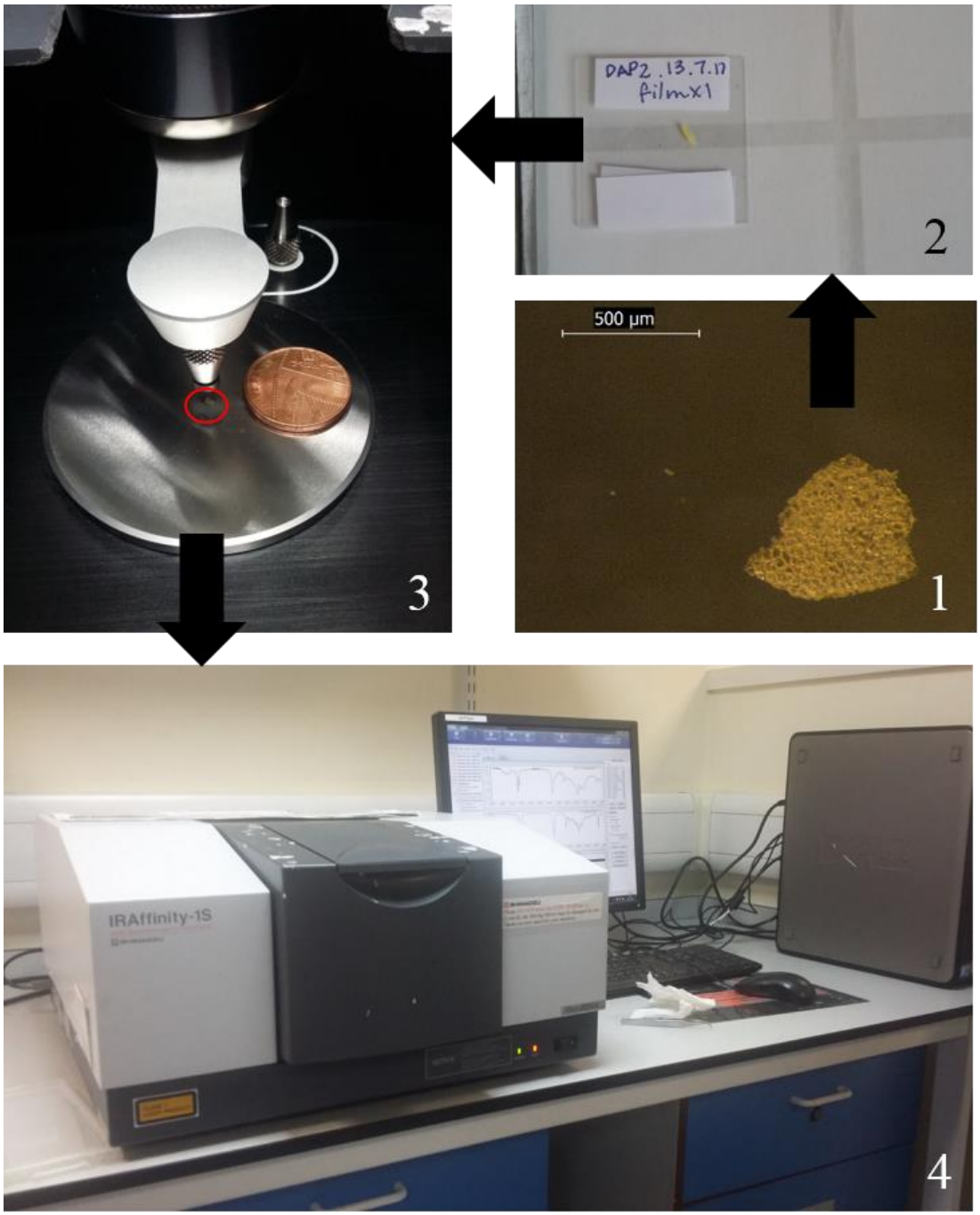


Figure C-5 Sample setup for analysis of suspected MPs by FTIR-ATR

Appendix D Electronic Supplementary Material (Blair et al. 2019a)

Table D-1 Visual (suspended and settled material) and chemical counts of suspected microplastics extracted from fractioned sediment samples collected from the River Kelvin on two sampling dates.

Visual counts, suspended

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
1	17/12/2015	0-5	2.80	24.95				0	1			1	1		1		2	
1	17/12/2015	0-5	2.00	24.77		2		2				0	3		3		5	
1	17/12/2015	0-5	1.40	24.99				0				0	2		2		2	
1	17/12/2015	0-5	1.00	24.97				0	1			1	2		2		3	
1	17/12/2015	0-5	0.71	24.97				0	5	1		6	1		1		7	
1	17/12/2015	0-5	0.50	25.00				0	2	4		6		1	1		7	
1	17/12/2015	0-5	0.36	25.03				0				0	3		3		3	
1	17/12/2015	0-5	0.25	24.97				0				0			0		0	
1	17/12/2015	0-5	0.18	14.31				0	1	4		5			0		5	
1	17/12/2015	0-5	0.13	3.74				0	1	3		4			0		4	
1	17/12/2015	0-5	0.09	1.01				0	1	3		4			0		4	
1	17/12/2015	0-5	0.06	0.56				0		2		2			0		2	
1	17/12/2015	0-5	<0.063	0.48				0		15		15			0		15	
1	17/12/2015	0-5	blk					0		1		1			0		1	
TOTAL					0	2	0	2	12	32	0	44	12	1	13	0	59	
1	17/12/2015	5-10	2.80	24.96				0				0	2		2		2	
1	17/12/2015	5-10	2.00	25.02		2		2				0	3		3	1	6	
1	17/12/2015	5-10	1.40	25.06		1		1				0	4		4	2	7	
1	17/12/2015	5-10	1.00	24.98				0		2		2			0		2	
1	17/12/2015	5-10	0.71	25.02				0		5		5	1		1		6	
1	17/12/2015	5-10	0.50	25.05				0				0			0		0	
1	17/12/2015	5-10	0.36	24.99				0				0			0		0	
1	17/12/2015	5-10	0.25	25.05				0				0			0	2	2	
1	17/12/2015	5-10	0.18	15.47				0		3		3			0		3	
1	17/12/2015	5-10	0.13	4.22				0				0			0		0	
1	17/12/2015	5-10	0.09	0.95				0		2		2			0		2	
1	17/12/2015	5-10	0.06	0.49				0		1		1			0		1	
1	17/12/2015	5-10	<0.063	0.48				0	5	2		7			0		7	
1	17/12/2015		blk					0		2		2			0		2	
TOTAL					0	3	0	3	5	15	0	20	10	0	10	5	38	
TOTAL BLANKS					0	0	0	0	0	3	0	3	0	0	0	0	3	
TOTAL SE1				441.49				5				64			23	5	97	220

Visual counts, suspended (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	0-2	2.80	0.23				0		1		1			0		1	
2	15/02/2016	0-2	2.00	0.11				0		5		5			0		5	
2	15/02/2016	0-2	1.40	0.52				0				0	1		1		1	
2	15/02/2016	0-2	1.00	2.22				0		1		1	1		1		2	
2	15/02/2016	0-2	0.71	7.04				0		3		3	2		2		5	
2	15/02/2016	0-2	0.50	24.71				0		1		1			0		1	
2	15/02/2016	0-2	0.36	28.94				0		1		1			0		1	
2	15/02/2016	0-2	0.25	9.36				0		6		6			0		6	
2	15/02/2016	0-2	0.18	1.53				0		3		3			0		3	
2	15/02/2016	0-2	0.13	0.36				0		3		3			0		3	
2	15/02/2016	0-2	0.09	0.08				0		4		4			0		4	
2	15/02/2016	0-2	0.06	0.05				0		2		2			0		2	
2	15/02/2016	0-2	<0.063	0.05				0		37		37			0		37	
2	15/02/2016	0-2	blk					0		1		1			0		1	
TOTAL					0	0	0	0	0	67	0	67	4	0	4	0	71	
2	15/02/2016	2-4	2.80	2.27				0				0			0		0	
2	15/02/2016	2-4	2.00	1.28				0				0			0		0	
2	15/02/2016	2-4	1.40	3.28				0				0			0		0	
2	15/02/2016	2-4	1.00	9.03				0		1		1			0		1	
2	15/02/2016	2-4	0.71	16.43				0		1		1		1	1		2	
2	15/02/2016	2-4	0.50	17.74				0				0	1		1		1	
2	15/02/2016	2-4	0.36	9.56				0				0			0		0	
2	15/02/2016	2-4	0.25	2.75				0				0			0		0	
2	15/02/2016	2-4	0.18	0.59				0		1		1			0		1	
2	15/02/2016	2-4	0.13	0.16				0		1		1			0		1	
2	15/02/2016	2-4	0.09	0.04				0				0			0		0	
2	15/02/2016	2-4	0.06	0.02				0				0			0		0	
2	15/02/2016	2-4	<0.063	0.01				0		6		6			0		6	
2	15/02/2016	2-4	blk					0				0			0		0	
TOTAL					0	0	0	0	0	10	0	10	1	1	2	0	12	

Visual counts, suspended (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	4-6	2.80	3.26				0			1	1			0		1	
2	15/02/2016	4-6	2.00	1.44				0		3		3			0		3	
2	15/02/2016	4-6	1.40	2.26				0				0			0		0	
2	15/02/2016	4-6	1.00	4.92				0				0			0		0	
2	15/02/2016	4-6	0.71	10.82				0				0			0		0	
2	15/02/2016	4-6	0.50	17.65				0		3		3			0		3	
2	15/02/2016	4-6	0.36	11.90				0				0			0		0	
2	15/02/2016	4-6	0.25	3.25				0		3		3		1	1		4	
2	15/02/2016	4-6	0.18	0.43				0				0			0		0	
2	15/02/2016	4-6	0.13	0.09				0		1		1			0		1	
2	15/02/2016	4-6	0.09	0.04				0		1		1			0		1	
2	15/02/2016	4-6	0.06	0.03				0		5		5			0		5	
2	15/02/2016	4-6	<0.063	0.01				0		3		3			0		3	
2	15/02/2016	4-6	blk					0				0			0		0	
TOTAL					0	0	0	0	0	19	1	20	0	1	1	0	21	
2	15/02/2016	6-8	2.80	1.51				0				0			0		0	
2	15/02/2016	6-8	2.00	0.47				0				0			0		0	
2	15/02/2016	6-8	1.40	1.91				0		1		1			0		1	
2	15/02/2016	6-8	1.00	6.49				0		1		1			0		1	
2	15/02/2016	6-8	0.71	13.50				0		1		1			0		1	
2	15/02/2016	6-8	0.50	18.80				0			1	1			0		1	
2	15/02/2016	6-8	0.36	12.49				0				0			0		0	
2	15/02/2016	6-8	0.25	3.99				0		1		1			0		1	
2	15/02/2016	6-8	0.18	0.66				0				0			0		0	
2	15/02/2016	6-8	0.13	0.13				0				0	1		1		1	
2	15/02/2016	6-8	0.09	0.04				0		2		2			0		2	
2	15/02/2016	6-8	0.06	0.02				0				0			0		0	
2	15/02/2016	6-8	<0.063	0.01				0		2		2			0		2	
2	15/02/2016	6-8	blk					0		2		2			0		2	
TOTAL					0	0	0	0	0	8	1	9	1	0	1	0	10	
TOTAL BLANKS					0	0	0	0	0	3	0	3	0	0	0	0	3	
TOTAL SE2				254.48				0				106			8	0	114	448

Visual counts, settled

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight (dry)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
1	17/12/2015	0-5	2.80	24.95				0				0			0		0	
1	17/12/2015	0-5	2.00	24.77	2	1	1	4				0	2	1	3		7	
1	17/12/2015	0-5	1.40	24.99		22		22				0	1		1		23	
1	17/12/2015	0-5	1.00	24.97	13	19		32				0	5	2	7		39	
1	17/12/2015	0-5	0.71	24.97	22	38		60				0	4	3	7		67	
1	17/12/2015	0-5	0.50	25.00	5	9		14				0	1	1	2		16	
1	17/12/2015	0-5	0.36	25.03	6	17		23				0		1	1		24	
1	17/12/2015	0-5	0.25	24.97	2	5		7				0			0		7	
1	17/12/2015	0-5	0.18	14.31		1		1				0		1	1		2	
1	17/12/2015	0-5	0.13	3.74				0				0			0		0	
1	17/12/2015	0-5	0.09	1.01				0				0			0		0	
1	17/12/2015	0-5	0.06	0.56				0				0			0		0	
1	17/12/2015	0-5	<0.063	0.48				0				0			0		0	
1	17/12/2015	0-5	blk					0				0			0		0	
TOTAL					50	112	1	163	0	0	0	0	13	9	22	0	185	
1	17/12/2015	5-10	2.80	24.96		1		1				0	1		1		2	
1	17/12/2015	5-10	2.00	25.02	2	5		7				0	2	1	3		10	
1	17/12/2015	5-10	1.40	25.06	5	3	2	10				0		1	1		11	
1	17/12/2015	5-10	1.00	24.98	8	17		25				0	3	3	6		31	
1	17/12/2015	5-10	0.71	25.02	29	25	4	58				0	6	6	12	1	71	
1	17/12/2015	5-10	0.50	25.05	10	16		26				0			0		26	
1	17/12/2015	5-10	0.36	24.99	11	8		19				0		2	2		21	
1	17/12/2015	5-10	0.25	25.05		1		1				0		2	2		3	
1	17/12/2015	5-10	0.18	15.47	1	1		2				0			0		2	
1	17/12/2015	5-10	0.13	4.22	1			1				0			0		1	
1	17/12/2015	5-10	0.09	0.95				0				0			0		0	
1	17/12/2015	5-10	0.06	0.49				0				0			0		0	
1	17/12/2015	5-10	<0.063	0.48				0				0			0		0	
1	17/12/2015							0				0			0		0	
TOTAL					67	77	6	150	0	0	0	0	12	15	27	1	178	
TOTAL SE1				441.49				313				0			49		363	822

Visual counts, settled (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	0-2	2.80	0.23				0				0			0		0	
2	15/02/2016	0-2	2.00	0.11				0				0			0		0	
2	15/02/2016	0-2	1.40	0.52				0				0			0		0	
2	15/02/2016	0-2	1.00	2.22				0				0			0		0	
2	15/02/2016	0-2	0.71	7.04	2			2				0	1		1		3	
2	15/02/2016	0-2	0.50	24.71	3	3		6				0			0		6	
2	15/02/2016	0-2	0.36	28.94		2		2				0			0		2	
2	15/02/2016	0-2	0.25	9.36		2		2				0			0		2	
2	15/02/2016	0-2	0.18	1.53				0				0			0		0	
2	15/02/2016	0-2	0.13	0.36				0				0			0		0	
2	15/02/2016	0-2	0.09	0.08				0				0			0		0	
2	15/02/2016	0-2	0.06	0.05				0				0			0		0	
2	15/02/2016	0-2	<0.063	0.05				0				0			0		0	
2	15/02/2016	0-2	blk					0				0			0		0	
TOTAL					5	7	0	12	0	0	0	0	1	0	1	0	13	
2	15/02/2016	2-4	2.80	2.27		1		1				0			0		1	
2	15/02/2016	2-4	2.00	1.28				0				0			0		0	
2	15/02/2016	2-4	1.40	3.28				0				0			0		0	
2	15/02/2016	2-4	1.00	9.03	1			1				0			0		1	
2	15/02/2016	2-4	0.71	16.43		2		2				0			0		2	
2	15/02/2016	2-4	0.50	17.74	2	1		3				0			0		3	
2	15/02/2016	2-4	0.36	9.56				0				0			0		0	
2	15/02/2016	2-4	0.25	2.75				0				0			0		0	
2	15/02/2016	2-4	0.18	0.59				0				0			0		0	
2	15/02/2016	2-4	0.13	0.16				0				0			0		0	
2	15/02/2016	2-4	0.09	0.04				0				0			0		0	
2	15/02/2016	2-4	0.06	0.02				0				0			0		0	
2	15/02/2016	2-4	<0.063	0.01				0				0			0		0	
2	15/02/2016	2-4	blk					0				0			0		0	
TOTAL					3	4	0	7	0	0	0	0	0	0	0	0	7	

Visual counts, settled (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	4-6	2.80	3.26				0				0			0		0	
2	15/02/2016	4-6	2.00	1.44				0				0			0		0	
2	15/02/2016	4-6	1.40	2.26		1		1				0			0		1	
2	15/02/2016	4-6	1.00	4.92				0				0			0		0	
2	15/02/2016	4-6	0.71	10.82	1	2		3				0			0		3	
2	15/02/2016	4-6	0.50	17.65	1			1				0			0		1	
2	15/02/2016	4-6	0.36	11.90	1	3		4				0			0		4	
2	15/02/2016	4-6	0.25	3.25		2		2				0			0		2	
2	15/02/2016	4-6	0.18	0.43				0				0			0		0	
2	15/02/2016	4-6	0.13	0.09				0				0			0		0	
2	15/02/2016	4-6	0.09	0.04				0				0			0		0	
2	15/02/2016	4-6	0.06	0.03				0				0			0		0	
2	15/02/2016	4-6	<0.063	0.01				0				0			0		0	
2	15/02/2016	4-6	blk					0				0			0		0	
TOTAL					3	8	0	11	0	0	0	0	0	0	0	0	11	
2	15/02/2016	6-8	2.80	1.51				0				0			0		0	
2	15/02/2016	6-8	2.00	0.47				0				0			0		0	
2	15/02/2016	6-8	1.40	1.91				0				0			0		0	
2	15/02/2016	6-8	1.00	6.49				0				0			0		0	
2	15/02/2016	6-8	0.71	13.50		1		1				0			0		1	
2	15/02/2016	6-8	0.50	18.80	1	2		3				0			0		3	
2	15/02/2016	6-8	0.36	12.49		1		1				0			0		1	
2	15/02/2016	6-8	0.25	3.99		3		3				0			0		3	
2	15/02/2016	6-8	0.18	0.66				0				0			0		0	
2	15/02/2016	6-8	0.13	0.13				0				0			0		0	
2	15/02/2016	6-8	0.09	0.04				0				0			0		0	
2	15/02/2016	6-8	0.06	0.02				0				0			0		0	
2	15/02/2016	6-8	<0.063	0.01				0				0		1	1		1	
2	15/02/2016	6-8	blk					0				0			0		0	
TOTAL					1	7	0	8	0	0	0	0	0	1	1	0	9	
TOTAL SE2				254.48				38				0			2		40	157

Chemical counts, SEM-EDS

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight (dry)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
1	17/12/2015	0-5	2.80	24.95				0	1			1			0		1	
1	17/12/2015	0-5	2.00	24.77				0				0		1	1		1	
1	17/12/2015	0-5	1.40	24.99				0				0		1	1		1	
1	17/12/2015	0-5	1.00	24.97				0	1			1			0		1	
1	17/12/2015	0-5	0.71	24.97				0	5	1		6			0		6	
1	17/12/2015	0-5	0.50	25.00				0	2	4		6		2	2		8	
1	17/12/2015	0-5	0.36	25.03				0				0			0		0	
1	17/12/2015	0-5	0.25	24.97				0				0			0		0	
1	17/12/2015	0-5	0.18	14.31				0	1	4		5			0		5	
1	17/12/2015	0-5	0.13	3.74				0	1	3		4			0		4	
1	17/12/2015	0-5	0.09	1.01				0	1	3		4			0		4	
1	17/12/2015	0-5	0.06	0.56				0		2		2			0		2	
1	17/12/2015	0-5	<0.063	0.48				0		15		15			0		15	
1	17/12/2015	0-5	blk					0		1		1			0		1	
TOTAL					0	0	0	0	12	32	0	44	0	4	4	0	48	
1	17/12/2015	5-10	2.80	24.96				0				0			0		0	
1	17/12/2015	5-10	2.00	25.02				0				0			0		0	
1	17/12/2015	5-10	1.40	25.06				0				0			0		0	
1	17/12/2015	5-10	1.00	24.98				0		2		2			0		2	
1	17/12/2015	5-10	0.71	25.02				0		5		5	1		1		6	
1	17/12/2015	5-10	0.50	25.05				0				0			0		0	
1	17/12/2015	5-10	0.36	24.99				0				0			0		0	
1	17/12/2015	5-10	0.25	25.05				0				0		2	2		2	
1	17/12/2015	5-10	0.18	15.47				0		3		3			0		3	
1	17/12/2015	5-10	0.13	4.22				0				0			0		0	
1	17/12/2015	5-10	0.09	0.95				0		2		2			0		2	
1	17/12/2015	5-10	0.06	0.49				0		1		1			0		1	
1	17/12/2015	5-10	<0.063	0.48				0	5	2		7			0		7	
1	17/12/2015							0		2		2			0		2	
TOTAL					0	0	0	0	5	15	0	20	1	2	3	0	23	
TOTAL SE1				441.49				0				64			7	0	71	161

Chemical counts, SEM-EDS (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	0-2	2.80	0.23				0		1		1			0		1	
2	15/02/2016	0-2	2.00	0.11				0		5		5			0		5	
2	15/02/2016	0-2	1.40	0.52				0				0			0		0	
2	15/02/2016	0-2	1.00	2.22				0		1		1			0		1	
2	15/02/2016	0-2	0.71	7.04				0		3		3			0		3	
2	15/02/2016	0-2	0.50	24.71				0		1		1			0		1	
2	15/02/2016	0-2	0.36	28.94				0		1		1			0		1	
2	15/02/2016	0-2	0.25	9.36				0		6		6			0		6	
2	15/02/2016	0-2	0.18	1.53				0		3		3			0		3	
2	15/02/2016	0-2	0.13	0.36				0		3		3			0		3	
2	15/02/2016	0-2	0.09	0.08				0		4		4			0		4	
2	15/02/2016	0-2	0.06	0.05				0		2		2			0		2	
2	15/02/2016	0-2	<0.063	0.05				0		37		37			0		37	
2	15/02/2016	0-2	blk					0		1		1			0		1	
TOTAL					0	0	0	0	0	67	0	67	0	0	0	0	67	
2	15/02/2016	2-4	2.80	2.27				0				0			0		0	
2	15/02/2016	2-4	2.00	1.28				0				0			0		0	
2	15/02/2016	2-4	1.40	3.28				0				0			0		0	
2	15/02/2016	2-4	1.00	9.03				0		1		1			0		1	
2	15/02/2016	2-4	0.71	16.43				0		1		1			0		1	
2	15/02/2016	2-4	0.50	17.74				0				0			0		0	
2	15/02/2016	2-4	0.36	9.56				0				0			0		0	
2	15/02/2016	2-4	0.25	2.75				0				0			0		0	
2	15/02/2016	2-4	0.18	0.59				0		1		1			0		1	
2	15/02/2016	2-4	0.13	0.16				0		1		1			0		1	
2	15/02/2016	2-4	0.09	0.04				0				0			0		0	
2	15/02/2016	2-4	0.06	0.02				0				0			0		0	
2	15/02/2016	2-4	<0.063	0.01				0		6		6			0		6	
2	15/02/2016	2-4	blk					0				0			0		0	
TOTAL					0	0	0	0	0	10	0	10	0	0	0	0	10	

Chemical counts, SEM-EDS (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	4-6	2.80	3.26				0			1	1			0		1	
2	15/02/2016	4-6	2.00	1.44				0		3		3			0		3	
2	15/02/2016	4-6	1.40	2.26				0				0		1	1		1	
2	15/02/2016	4-6	1.00	4.92				0				0			0		0	
2	15/02/2016	4-6	0.71	10.82				0				0			0		0	
2	15/02/2016	4-6	0.50	17.65				0		3		3			0		3	
2	15/02/2016	4-6	0.36	11.90				0				0			0		0	
2	15/02/2016	4-6	0.25	3.25				0		3		3		1	1		4	
2	15/02/2016	4-6	0.18	0.43				0				0			0		0	
2	15/02/2016	4-6	0.13	0.09				0		1		1			0		1	
2	15/02/2016	4-6	0.09	0.04				0		1		1			0		1	
2	15/02/2016	4-6	0.06	0.03				0		5		5			0		5	
2	15/02/2016	4-6	<0.063	0.01				0		3		3			0		3	
2	15/02/2016	4-6	blk					0				0			0		0	
TOTAL					0	0	0	0	0	19	1	20	0	2	2	0	22	
2	15/02/2016	6-8	2.80	1.51				0				0			0		0	
2	15/02/2016	6-8	2.00	0.47				0				0			0		0	
2	15/02/2016	6-8	1.40	1.91				0		1		1			0		1	
2	15/02/2016	6-8	1.00	6.49				0		1		1			0		1	
2	15/02/2016	6-8	0.71	13.50				0		1		1			0		1	
2	15/02/2016	6-8	0.50	18.80				0			1	1			0		1	
2	15/02/2016	6-8	0.36	12.49				0				0			0		0	
2	15/02/2016	6-8	0.25	3.99				0		1		1			0		1	
2	15/02/2016	6-8	0.18	0.66				0				0			0		0	
2	15/02/2016	6-8	0.13	0.13				0				0		2	2		2	
2	15/02/2016	6-8	0.09	0.04				0		2		2			0		2	
2	15/02/2016	6-8	0.06	0.02				0				0			0		0	
2	15/02/2016	6-8	<0.063	0.01				0		2		2			0		2	
2	15/02/2016	6-8	blk					0		2		2			0		2	
TOTAL					0	0	0	0	0	8	1	9	0	2	2	0	11	
TOTAL SE2				254.48				0				106			4	0	110	432

Appendix E Electronic Supplementary Material (Blair et al. 2019b)

Daily discharge at the WWTP

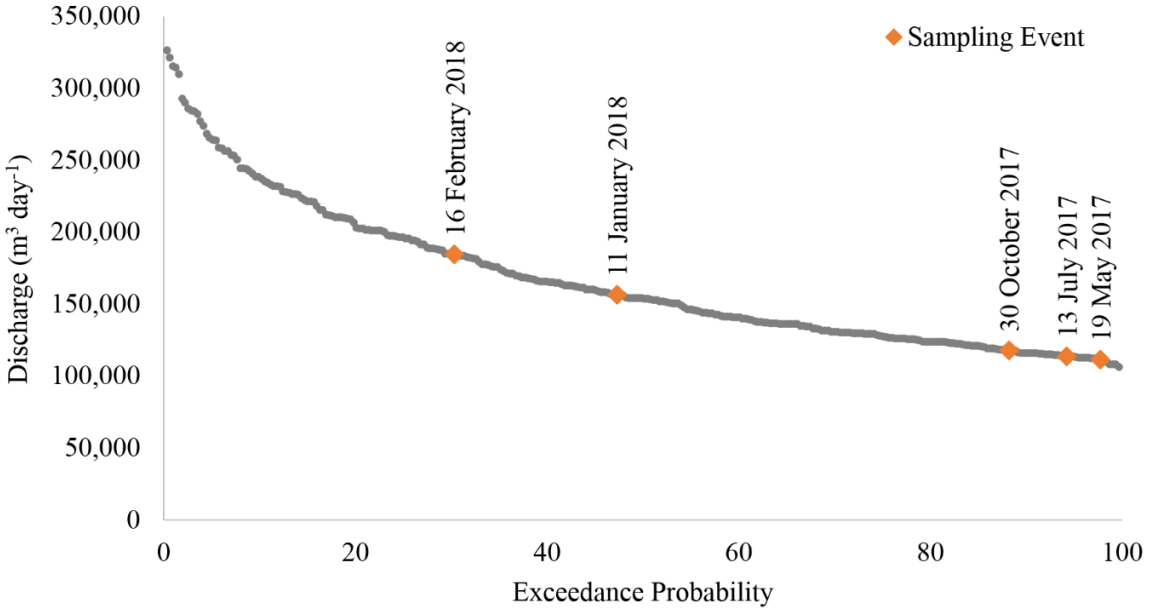


Figure E-1 Flow duration curve for the tertiary wastewater treatment plant based on daily inlet flows during the period of study from May 2017 to February 2018. Contains Scottish Water data © 2018. All rights reserved.

Quality Control

A) Blanks

Two types of blank controls were used:

1) Procedural blanks: one blank was created for each sampling event by running 5 L of DI water through the same sample equipment used to collect samples, and then processed the same way as wastewater. The purpose of the procedural blanks was to evaluate possible cross-contamination from generation of particles from plastic equipment used during sampling – these include plastic bottles, synthetic ropes, and a plastic funnel.

2) Laboratory blanks: blanks were created in triplicates by placing 1 L of DI water in the same glass containers used for sample processing and leaving uncovered on lab benches during the extraction process, then filtering in parallel with each run of field samples. The purpose of the lab blanks was to capture cross contamination from deposition of airborne particles in the general environment.

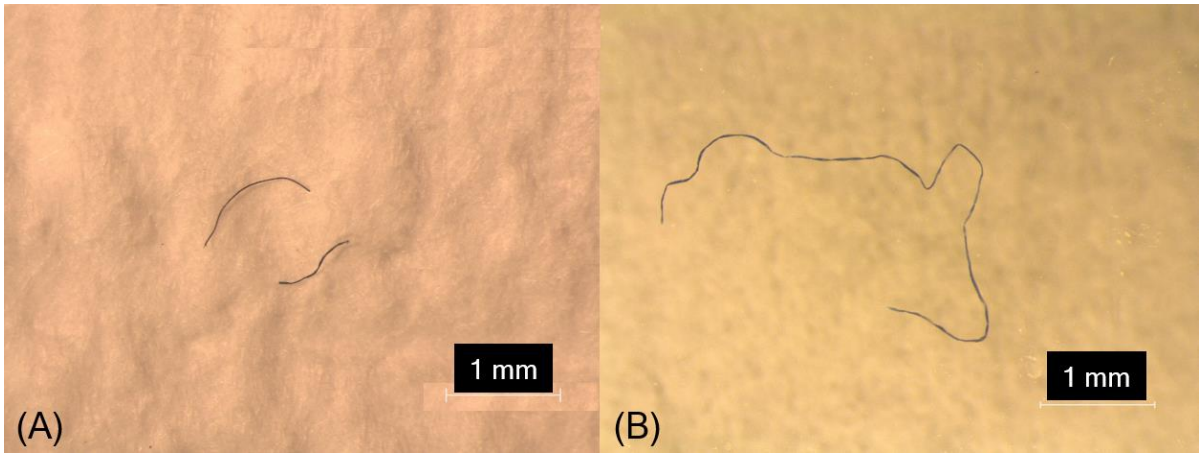


Figure E-2 Representative examples of coloured fibres observed in lab (A) and procedural (B) blanks. No other types of particles were identified.

Table E-1 Fibre counts for individual procedural (n=1) and lab blanks (n=3) during each sampling event. Lab blanks are not available for the first sampling event (SE1)

Sampling Event	Blank	Fibre Counts*	Abundance (items L^{-1})
1	Procedural	14	3
2	Procedural	11	2
	Lab1	2	2
	Lab2	3	3
	Lab3	1	1
3	Procedural	13	3
	Lab1	2	2
	Lab2	3	3
	Lab3	0	0
4	Procedural	4	1
	Lab1	1	1
	Lab2	2	2
	Lab3	0	0
5	Procedural	7	1
	Lab1	3	3
	Lab2	1	1
	Lab3	0	0

* Coloured fibres were the only type of particles observed in procedural and lab blanks.

B) Fragmentation tests

Fragmentation tests using MP-spiked DI water were carried out to assess if the extraction process could generate secondary MPs at various stages. For this, 12 spiked samples were created by placing 500 mL of DI water and 10 standard PE beads each in glass Erlenmeyer flasks. Nine of the spiked samples were treated with 30% H_2O_2 (1:1, v/v) and three left as blank controls (no treatment). Three samples were extracted under vacuum filtration as described above, before and after each step of the WPO treatment: (1) no treatment, (2) after H_2O_2 addition, (3) after heating, (4) after stirring. The filters were examined under light microscopy for quantification of whole beads and fragmented pieces. Production of fragments (range 1-18, mean 6, median 4, standard deviation 6) was observed (**Fig S3**; **Table S2**) in half of spiked samples after heating (1 fragment in one replicate only) and after stirring (6 and 21 fragments in two replicates), indicating that the extraction protocol may contribute to the breakdown of MP pieces already present in the samples and could result in higher fragment counts and a misleading interpretation of their abundance.

Recovery rates were assessed from fragmentations tests with DI standards and spiking of wastewater samples with 50 standard beads. Whole bead recovery was 27-48 out of 50 beads (mean 42, median 43, standard deviation 5) in spiked wastewater samples (n=3), and 6-10 out of 10 beads (mean 9, median 9, standard deviation 1) in DI standards (**Table S2**). As these validation tests were conducted with standard beads, they represent types of particles that were almost negligible in this study, thus recovery and fragmentation data were not used in correction of sample data. However, controlled quality assurance tests are reported here as they should be part of routine testing for validation of results, particularly as research progresses towards method standardisation.

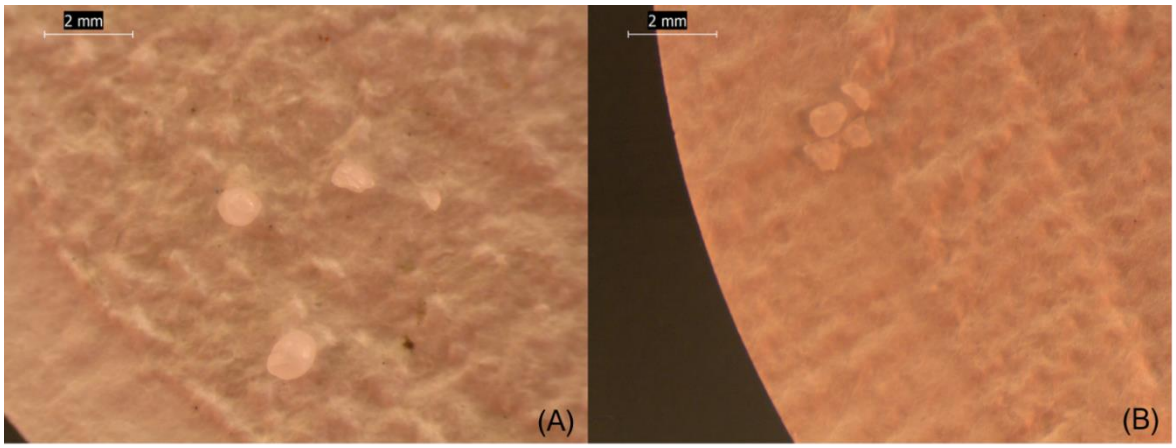


Figure E-3 Examples of whole PE microbeads (A) and bead fragments (A-B) observed during fragmentation tests.

Table E-2 Bead recovery and fragmentation at different steps of the extraction process: **H_2O_2**, after H_2O_2 addition; **+Heat**, after H_2O_2 and heating in water bath at 70°C for 30 minutes; **+Stir**, after H_2O_2 addition and heating in water bath immediately followed by magnetic stirring during 10 minutes. **Blank** controls received no treatment and were extracted at the end of the process (i.e. three days)

Sample ID	DI Water (mL)	H_2O_2 (mL)	Beads Added (n)	Beads Recovered (n)	% Recovery	Fragments
Blank	500	0	10	9	90	0
Blank	500	0	10	10	100	0
Blank	500	0	10	9	90	0
H_2O_2	500	500	10	7	70	0
H_2O_2	500	500	10	10	100	0
H_2O_2	500	500	10	9	90	0
+Heat	500	500	10	10	100	0
+Heat	500	500	10	9	90	0
+Heat	500	500	10	8	80	1
+Stir	500	500	10	10	100	0
+Stir	500	500	10	6	60	21
+Stir	500	500	10	7	70	6

FTIR-ATR Characterisation

Criteria and Rationale

Confirmation of plastics was conducted by Fourier-transform infrared-attenuated total reflectance spectroscopy (FTIR-ATR), using a Shimadzu IRAffinity-1S FTIR with diamond crystal and 20 scans. Materials were identified by comparing the unknown spectra to those in the Shimadzu LabSolutions IR libraries, which contain approximately 12,000 reference spectra. For each particle, the top three automated matches were assessed visually for accuracy and a minimum score of 700 (maximum 1000) was deemed acceptable, below which particles were considered unknown. Except for four pieces (**Table S3**), the highest score was considered acceptable and reported. While manual validation is important for verification, it was considered that there is likely to be an error involved with the manual interpretation as well and thus the decision was made to follow a consistent approach that would provide robustness. The focus with this approach was to improve transferability across studies by providing a baseline that would generate the same result to someone else if they were to analyse the same particle, as doing a manual interpretation may be subjective and lead to different findings.

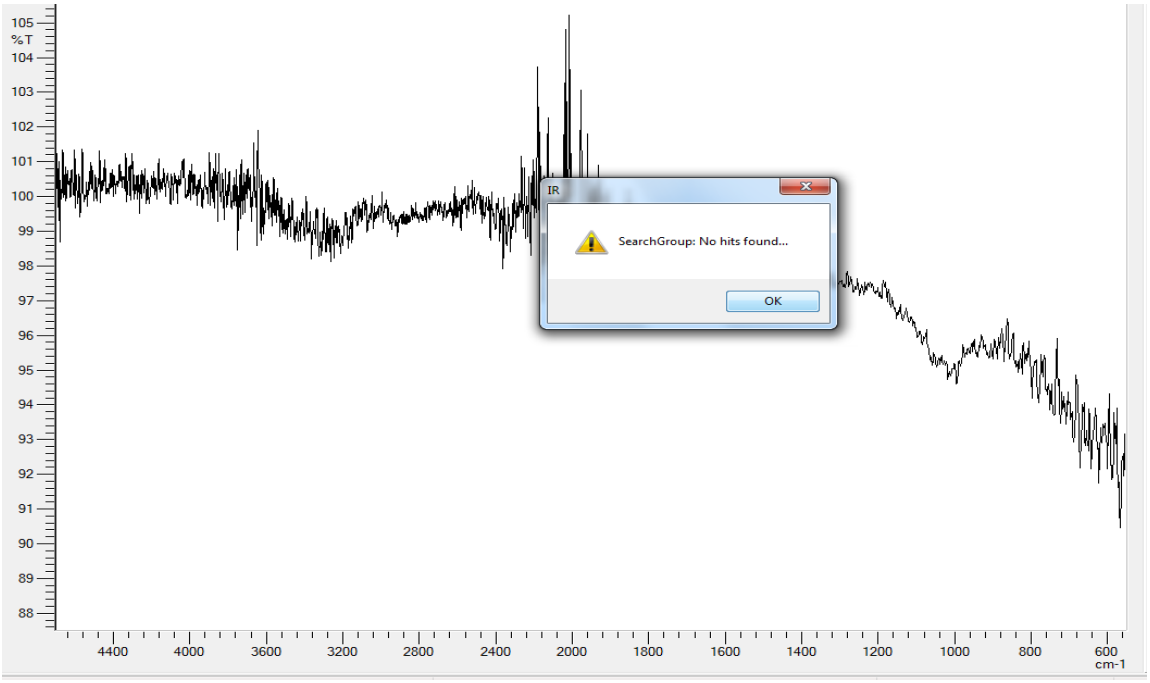


Figure E-4 Example of unidentifiable spectrum generated by FTIR-ATR analysis

Table E-3 Match scores for secondary-type particles (n=70) of size 300-2800 µm analysed by FTIR-ATR

#	Type	Top Score	Automated Matches		
			1	2	3
1	Film	751	Lecithin	Butter	Margarine
2	Film	769	Lecithin	Butter	PVS
3	Film	769	Cellulose (paper)	Tencel	Cellulose (cotton)
4	Film	743	Cellulose (paper)	Tencel	Cellulose (Bemberg)
5	Film	737	Tencel	Cellulose (Bemberg)	Cellulose (paper)
6	Film	747	Cellulose (paper)	Tencel	Cellulose (Bemberg)
7	Film	764	Cellulose (paper)	Tencel	Cellulose (Bemberg)
8	Film	762	Cellulose (paper)	Lecithin	Tencel
9	Film	806	Lecithin	Cooking oil	Margarine
10	Film	722	Cellulose (paper)	Tencel	Polyacetylene
11	Film	789	Lecithin	Butter	Olive oil
12	Film	724	Cellulose (paper)	Cellulose (cotton)	Cellulose (Ramie)
13	Fragment	<700	unknown		
14	Fibre	712	PP	PP	none
15	Fibre	725	PP	PP	none
16	Fibre	795	PP	PP	PB
17	Film	790	Lecithin	Margarine	Butter
18	Film	745	Cooking oil	Lecithin (730)*	Acrylic adhesive
19	Film	732	Cellulose (paper)	Cellulose (cotton)	Cellulose (Ramie)
20	Film	779	PVS	Lecithin	Cooking oil
21	Film	768	Ethylene_ EthylAcrylate	PVS	Lecithin
22	Fibre	<700	Unknown		
23	Fibre	<700	Unknown		
24	Fibre	<700	Unknown		
25	Film	724	Lecithin	Ca Stereate	PE-PP
26	Film	810	Cooking oil	PVS (808)*	Lecithin
27	FIlm	733	Cellulose (paper)	Cellulose (cotton)	Cellulose (Ramie)
28	Fragment	868	POM	POM	POM
29	Fragment	775	PP	PP	Paraffin
30	Fragment	811	PP	PP	PB
31	Fragment	763	PP	PP	none
32	Fibre	920	PE	HDPE	EAA
33	Fibre	763	PP	PP	none
34	Fibre	738	Cellulose (paper)	Tencel	Cellulose (Bemberg)
35	Fibre	760	PP	PP	
36	Fibre	765	PP	PP	
37	Film	703	Protein		
38	Fibre	730	PP	PP	
39	Fibre	703	PP	none	
40	Film	809	PE	HDPE	PE oxidized
41	Fragment	863	Paraffin	PE+PP (838)*	PP
42	Film	817	Lecithin	Cooking oil	PVS

#	Type	Top Score	Automated Matches		
			1	2	3
43	Fragment	826	PP	PP	Paraffin
44	Film	810	Cellulose (paper)	Cellulose (cotton)	Cellulose (Ramie)
45	Film	756	Cellulose (paper)	Cellulose (cotton)	Cellulose (Bemberg)
46	Film	<700	Unknown		
47	Film	758	Cellulose (paper)	Lecithin	Margarine
48	Film	857	PE oxidized	EEA	PE
49	Fibre	893	Cellulose (cotton)	Cellulose (Ramie)	Cellulose (paper)
50	Film	771	Cellulose (paper)	Margarine	Cellulose (Bemberg)
51	Film	780	Tencel	Cellulose (Bemberg)	Cellulose (paper)
52	Film	842	PVS	EEA	PE oxidized
53	Film	760	Lecithin	Cooking oil	Butter
54	Film	771	Cellulose (paper)	Tencel	Cellulose (Bemberg)
55	Fibre	778	PP	PP	PB
56	Fibre	782	PP	PP	
57	Fragment	752	PP	PP	
58	Fibre	795	PP	PP	
59	Film	841	Cellulose (cotton)	Cellulose (Ramie)	Cellulose (paper)
60	Fibre	<700	Unknown		
61	Film	842	Cellulose (paper)	Cellulose (cotton)	Cellulose (Ramie)
62	Fragment	<700	Unknown		
63	Fragment	775	PVS	Cooking oil	Lecithin
64	Film	768	Cellulose (Bemberg)	Lecithin	Tencel
65	Film	771	Cellulose (paper)	Cellulose (cotton)	Cellulose (Ramie)
66	Fibre	<700	Unknown		
67	Film	750	Cooking oil	Lecithin	PVS (744)*
68	Film	768	Cellulose (paper)	Cellulose (cotton)	Cellulose (Ramie)
69	Film	756	Tencel	Cellulose (paper)	Cellulose (Bemberg)
70	Film	722	Cellulose (Bemberg)	Protein (soybean powder)	Tencel

* Selected match other than top score

Appendix F Contamination controls for the River Clyde study

Table F-1 Fibre counts in atmospheric lab blanks filtered in parallel with sediment and water extraction runs. No other type of particles were observed.

Date	Sample ID	Particle Count (n)	
		Fibre, pale	Fibre, coloured
02-Aug-18	Blank Run 1	0	2
	Blank Run 2	0	1
	Blank Run 3	0	1
	Blank Run 4	1	0
	Blank Run 5	0	0
23-Aug-18	Blank Run 1	0	0
	Blank Run 2	0	3
	Blank Run 3	0	1
	Blank Run 4	0	1
	Blank Run 5	0	0
07-Nov-18	Blank Run 1	0	2
	Blank Run 2	0	0
	Blank Run 3	2	0
	Blank Run 4	NA	NA
	Blank Run 5	0	2
22-Jan-19	Blank Run 1	0	0
09-Apr-19	Blank Run 1	0	3
03-Jun-19	Blank Run 1	0	9

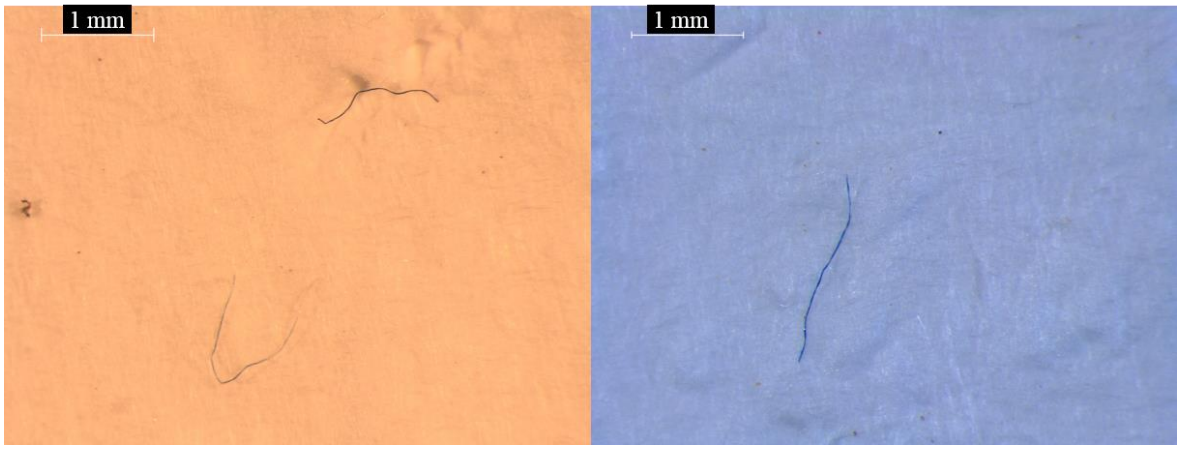
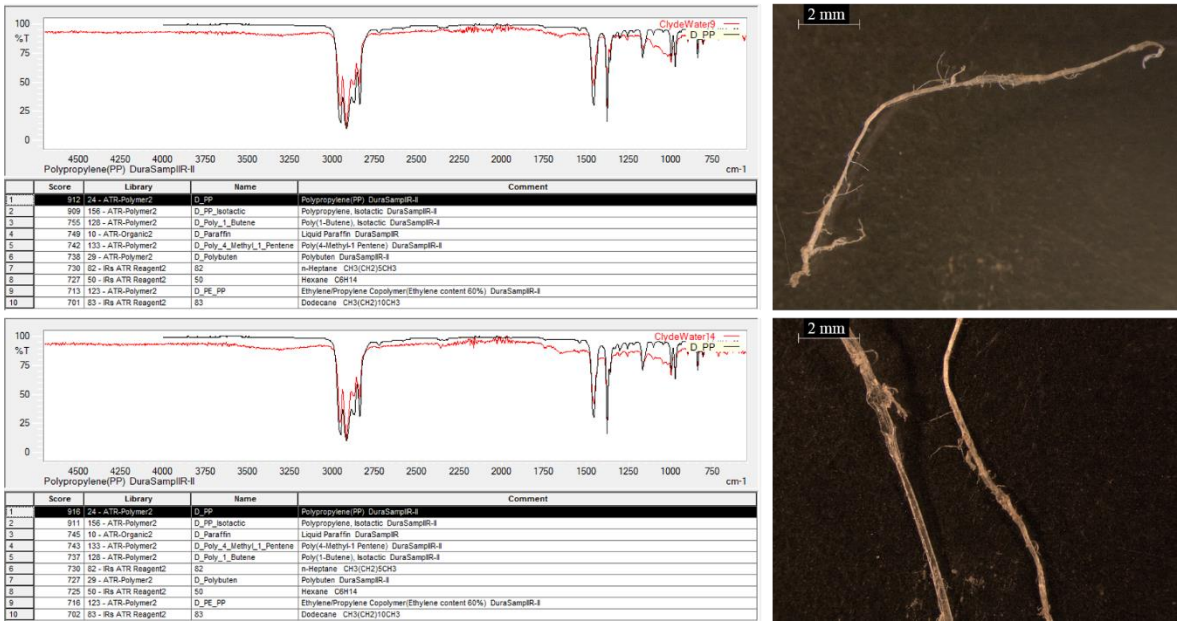


Figure F-1 Examples of common fibres observed in atmospheric laboratory blanks



	Score	Library	Name	Comment
1	912	24 - ATR-Polymer2	D_PP	Polypropylene(PP) DuraSamplIR-II
2	909	156 - ATR-Polymer2	D_PP_Isotactic	Polypropylene, Isotactic DuraSamplIR-II
3	755	128 - ATR-Polymer2	D_Poly_1_Butene	Poly(1-Butene), Isotactic DuraSamplIR-II
4	749	10 - ATR-Organic2	D_Paraffin	Liquid Paraffin DuraSamplIR
5	742	133 - ATR-Polymer2	D_Poly_4_Methyl_1_Pentene	Poly(4-Methyl-1 Pentene) DuraSamplIR-II
6	738	29 - ATR-Polymer2	D_Polybuten	Polybuten DuraSamplIR-II
7	730	82 - IRs ATR Reagent2	82	n-Heptane CH3(CH2)5CH3
8	727	50 - IRs ATR Reagent2	50	Hexane C6H14
9	713	123 - ATR-Polymer2	D_PE_PP	Ethylene/Propylene Copolymer(Ethylene content 60%) DuraSamplIR-II
10	701	83 - IRs ATR Reagent2	83	Dodecane CH3(CH2)10CH3

	Score	Library	Name	Comment
1	916	24 - ATR-Polymer2	D_PP	Polypropylene(PP) DuraSamplIR-II
2	911	156 - ATR-Polymer2	D_PP_Isotactic	Polypropylene, Isotactic DuraSamplIR-II
3	745	10 - ATR-Organic2	D_Paraffin	Liquid Paraffin DuraSamplIR
4	743	133 - ATR-Polymer2	D_Poly_4_Methyl_1_Pentene	Poly(4-Methyl-1 Pentene) DuraSamplIR-II
5	737	128 - ATR-Polymer2	D_Poly_1_Butene	Poly(1-Butene), Isotactic DuraSamplIR-II
6	730	82 - IRs ATR Reagent2	82	n-Heptane CH3(CH2)5CH3
7	727	29 - ATR-Polymer2	D_Polybuten	Polybuten DuraSamplIR-II
8	725	50 - IRs ATR Reagent2	50	Hexane C6H14
9	716	123 - ATR-Polymer2	D_PE_PP	Ethylene/Propylene Copolymer(Ethylene content 60%) DuraSamplIR-II
10	702	83 - IRs ATR Reagent2	83	Dodecane CH3(CH2)10CH3

Figure F-2 Chemical and visual characteristics of common fibres in River Clyde water samples (top) and sampling rope fibres (bottom).

Appendix G Visual comparisons of spatial samples in the River Clyde study

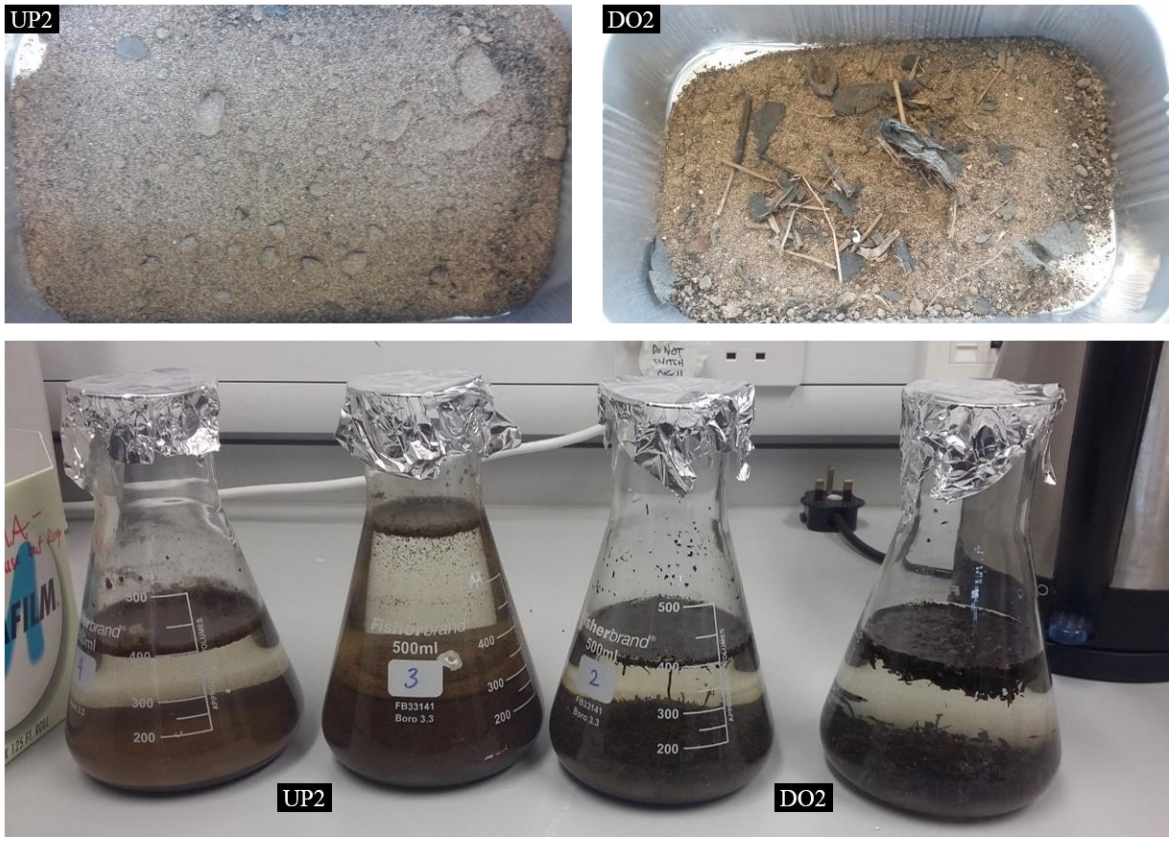


Figure G-1 Visual comparison of upstream and downstream sediment after oven-drying at 80°C (top) and during density separation (bottom).

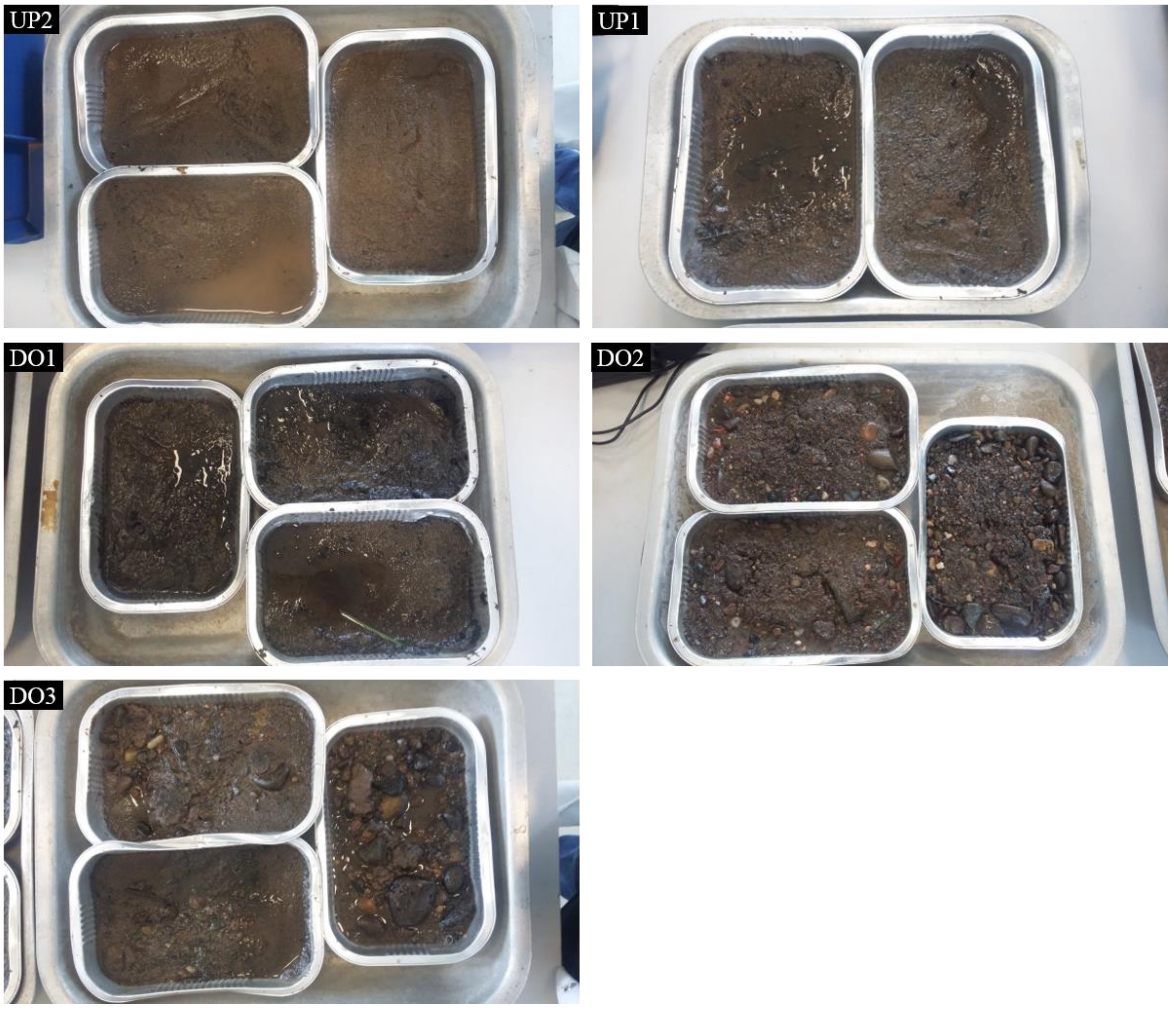


Figure G-2 Visual comparison of wet spatial sediment samples collected August 2, 2018 in the River Clyde. UP, upstream; DO, downstream

Appendix H Relationship between water quality parameters and MP abundance

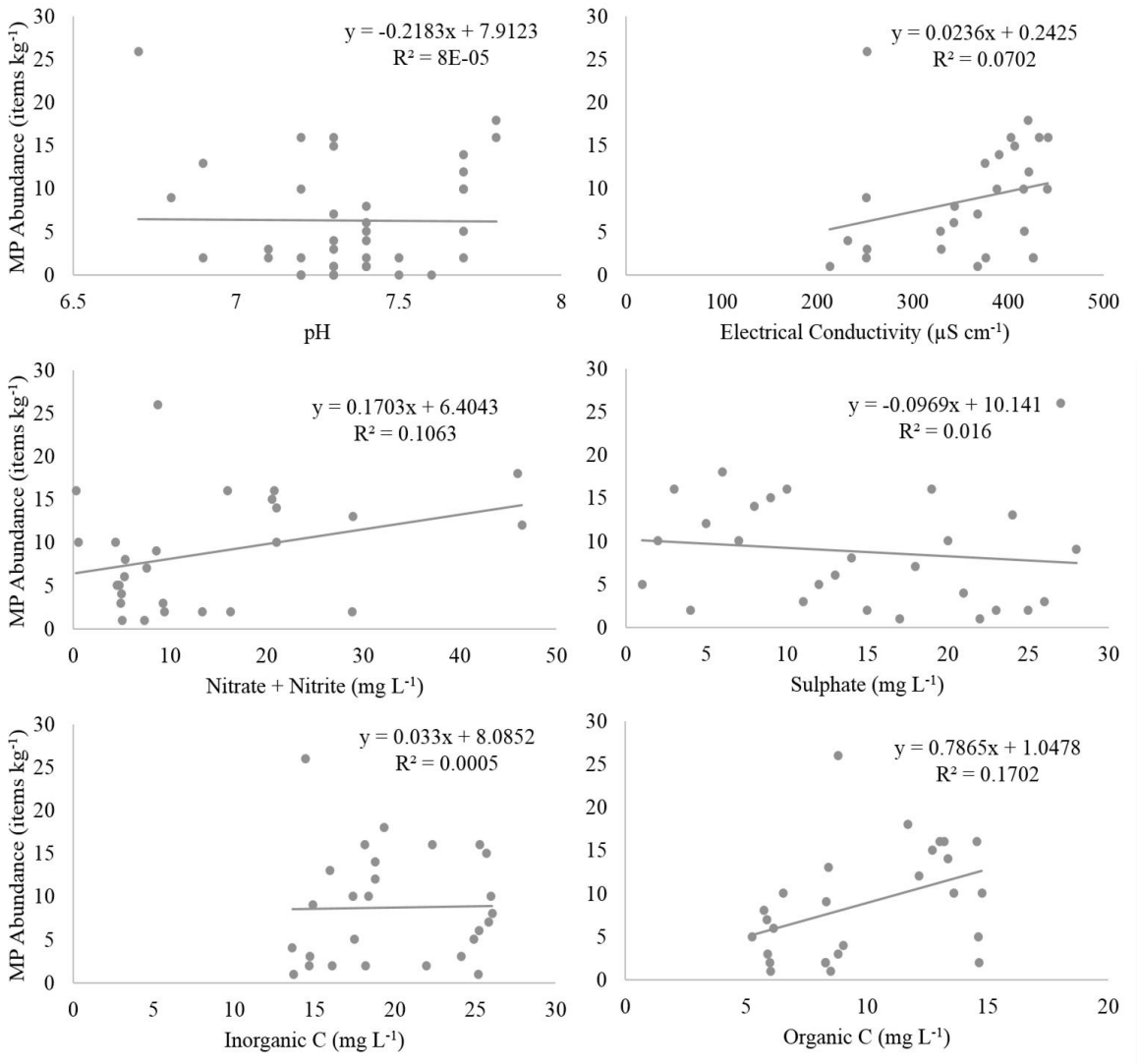


Figure H-1 Correlation between MP abundance (items kg^{-1}) and water parameters